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CONTRACTING ORGANIZATION: Vaccine and Immunology Research Institute
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The 9th National Symposium on Basic Aspects of Vaccines was held 30 Apr 03 - 2 May 03. There were approximately 250 participants. 23 Speakers presented information on "Interface Between Innate and Adaptive Immunity", "Bioterrorism Vaccines", "Bacterial Vaccines", "In Vivo Cell Interaction and Trafficking", and "Vaccines Against Allergy and Asthma". In addition, approximately 50 participants presented work via poster presentation.

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9th National Symposium: Basic Aspects of Vaccines

April 30 – May 2, 2003

9th National Symposium
BASIC ASPECTS OF VACCINES

April 30 – May 2, 2003

Sponsored by the Walter Reed Army Institute of Research
Silver Spring, MD

Wednesday, 30 April 2003

0700 - 0815 Registration and Poster Set-Up

0815 - 0830 Presentation of the Colors by the Walter Reed Army Institute of Research and Naval Medical Research Center Color Guard (Soloist: Ms. Detra Sparrow)
Welcoming address by COL Charles E. McQueen, MC, Commander, Walter Reed Army Institute of Research

SCIENTIFIC PROGRAM

Minisymposium I: Interface between Innate and Adaptive Immunity
(Mitchell Kronenberg, Chair)

0830- 0910 **Jacques Banchereau (Baylor Institute)**
Dendritic Cells as Components of Both Innate and Adaptive Immunity

0910-0920 Discussion

0920-1000 **Yong-jun Liu (MD Anderson Cancer Center)**
Regulation of the Anti-Viral Immune Response and Allergic Responses by Dendritic Cells

1000-1010 Discussion

1010-1050 Coffee Break and Poster Viewing

1050-1130 **David Underhill (Systems Biology)**
Microbial Recognition by Toll-Like Receptors and Friends

1130-1140 Discussion

1140-1220 **Mitchell Kronenberg (La Jolla Institute)**
Lymphocyte Responses on Demand: NKT Cells Combine Features of Innate and Adaptive Immunity

1220-1230 Discussion

1230-1330 **Lunch Break**

1040-1120 **George Siber (Wyeth)**
Bacterial Conjugate Vaccines
1120-1130 Discussion

1130-1210 **Myron (Mike) Levine (Univ of Maryland)**
Update on Vaccines Against Bacterial Enteric Infections
1210-1220 Discussion

1230-1330 **Lunch Break**

Minisymposium VI: In Vivo Cell Interaction and Trafficking *(Ronald Germain, Chair)*

1330-1410 **Marc Jenkins (Univ of Minnesota)**
Imaging Cell Interactions in Lymph Nodes
1410-1420 Discussion

1420-1500 **Ronald Germain (NIAID, NIH)**
In Vivo Analysis of the Activation of CD4 T Cells
1500-1510 Discussion

1510-1550 Coffee Break and Poster Viewing

1550-1630 **Ulrich Von Andrian (Center for Blood Research)**
In Vivo Analysis of T Cell Migration To and Within Lymph Nodes
1630-1640 Discussion

1640-1720 **Christopher Contag (Stanford)**
Tracking Immune Cells in Cancer and Autoimmunity
1720-1730 Discussion

Friday, 2 May 2003

Minisymposium V: Vaccines Against Allergies and Asthma
(Dale Umetsu, Chair)

0830- 0910 **Barry Kay (Imperial College of Medicine)**
Cat Peptide Vaccines for Allergy
0910-0920 Discussion

0920-1000 **Robert Coffman (Dynavax)**
CpG Conjugate Vaccines for Allergy
1000-1010 Discussion

1010-1040 Coffee Break and Poster Viewing

I

Interface Between Innate and Adaptive Immunity
(Mitchell Kronenberg, Chair)

Microbial Recognition by Toll-like receptors and Friends.

David Underhill, PhD, Institute for Systems Biology, Seattle, WA

The innate immune system uses a wide variety of receptors to identify potentially harmful microbes by specifically recognizing conserved structural motifs shared by many microbes, but not found in self tissues. The Toll-like receptors are a family of innate immune recognition receptors that play a central role in the induction of inflammatory responses to microbes by recognizing diverse structures including lipopolysaccharides, lipoproteins, flagellin, and bacterial DNA. We have used cDNA microarray technology to characterize transcriptional responses in macrophages stimulated by a variety of Toll-like receptor agonists and microbes. The data identify specific cases in which Toll-like receptor signaling cannot account for the spectrum of observed responses suggesting that additional pattern recognition receptors collaborate with TLRs. We have characterized how microbial recognition and signaling by a new receptor called Dectin-1, a lectin family receptor for ?-glucans, collaborates with TLRs in shaping inflammatory and antimicrobial responses in macrophages and dendritic cells.

II

Bioterrorism Vaccines
(Gary Nabel and Barney Graham, Co-Chairs)

Nancy Sullivan	**Ebola Virus Vaccines and Pathogenecity**
Lewis McCurdy	**Immunogenecity of Alternative Smallpox Vaccines**
Peter Palese	**Innate Immunity and Influenza Virus Infection**
Arthur Friedlander	**Anthrax Vaccines: The Next Generation**

GENETIC ENGINEERING OF RNA VIRUSES: TOWARD BETTER VACCINES

Peter Palese, Ph.D.
Department of Microbiology
Mount Sinai School of Medicine

Despite the extraordinary successes of vaccines developed over the last 100 years, there is still much to be done to make existing vaccines better and safer and to develop novel vaccines for diseases such as AIDS and malaria and for infectious agents that could be used against bioterrorism. I will focus on the development of reverse genetics techniques for negative-strand RNA viruses. Such techniques have made it possible to do detailed structure-function studies of specific viral genes and to better understand the virulence/pathogenicity of these viruses. In the near future, it is expected that these efforts should lead to improved influenza virus vaccines and to the development of Newcastle disease virus as a vaccine platform for use against a variety of infectious diseases.

Anthrax Vaccines: The Next Generation

Arthur M. Friedlander, U.S. Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702-5011

Anthrax, caused by Bacillus anthracis, is an ancient disease of domesticated animals and humans that has long been considered a potential biological weapon. The revelation that B. anthracis was weaponized before the Gulf War of 1990 led to the vaccination of the Armed Forces, representing the first time in history that humans were vaccinated to protect against a biological weapon. The successful use of B. anthracis as a bioterrorist agent in the fall of 2001 confirmed our worst fears and changed the practice of medicine. In this presentation, I will review aspects of the biology and pathogenesis of the organism as it relates to vaccines, the historical approaches to vaccination, and the status of research on new vaccines as well as alternative adjuvant and delivery systems.

III

Bacterial Vaccines
(George Siber, Chair)

Rino Rappuoli	**A Genome-based Vaccine Approach to Menningococcus B**
Richard Moxon	**Candidacy of LPS as a Vaccine Against Group B Meningococcal Disease**
George Siber	**Bacterial Conjugate Vaccines**
Myron (Mike) Levine	**Update on Vaccines Against Bacterial Enteric Infections**

Meeting	9th National Symposium on Basic Aspects of Vaccines
Lecture title	Candidacy of inner core LPS as a vaccine against non-typeable *Haemophilus influenzae* and Serogroup B *Neisseria meningitidis*
Lecturer name	Richard Moxon (on behalf of University of Oxford, UK and Department of Biological Chemistry, National Research Council, Ottawa, Canada)
Major Points	1. Rationale for vaccines to prevent infections caused by non-typeable *Haemophilus influenzae* and Serogroup B *Neisseria meningitidis*. 2. Problem of alternative approaches to capsular polysaccharide conjugate vaccines and reasons for the need to take a different approach. 3. Rationale of inner core lipopolysaccharide (LPS) vaccine as a candidate 4. Evidence that inner core LPS epitopes are conserved, accessible to antibodies and capable of inducing protective immunity 5. A novel conjugation strategy 6. Future prospects
Abstract	Safe and highly effective vaccines to prevent invasive diseases caused by *Haemophilus influenzae* (*Hi*) type b and Serogroup C *Neisseria meningitidis* (*Nm*) are licensed and have been incorporated into routine immunisation programmes in some countries. There are currently no licences vaccines available for the prevention of diseases caused by non-typeable (capsule-deficient) *Hi or* Serogroup B *strains of Nm in infants.* Vaccines for non-typeable (NT) *Hi* and *Nm*B that are not based on inducing anti-capsular antibodies are needed. Lipopolysacharide (LPS), the major surface antigen of the bacterial cell envelope, consists of membrane-anchored lipid A to which several core sugars and other substituents, such as phosphethanolamine, are added to result in a surface expressed macromolecule. The sugars are relatively conserved for the LPS inner core but are very variable on the outer core. Our research has investigated the candidacy of inner core epitopes as potental vaccine candidate antigens. We have used genomics, classical genetics, structural analyses and immunobiology to determine how conserved the inner core LPS (icLPS) structures are in strains associated with carriage and disease, their accessibility to host immune responses (antibodies) and their potential to elicit protection *in vitro* and *in vivo.* Using *Nm*B as an exemplar, we have found that monoclonal antibodies can protect against some but not all invasive *Nm*B strains in infant rats. Based on these results, we have made glycolipid icLPS -CRM_{197} conjugates. These conjugates elicit a specific IgG response in mice and rabbits and, in some mice, there is evidence of functional activity *in vitro* and *in vivo.* These preliminary results are encouraging but further work is needed to provide the compelling evidence required to justify future studies in phase 1 clinical trials of humans.

References	1. Zollinger. New and Improved Vaccines against Meningococcal Disease. New Generation Vaccines. 2nd. Edition. Edited by Levine, Woodrow, Kaper and Cobon. 1997. Marcel Dekker. Pp. 469-468 2. Plested JS, Makepeace K, Jennings MP, Gidney MA, Lacelle S, Brisson JR, Cox AD, Martin A, Bird AG, Tang CM, Mackinnon FM, Richards JC and Moxon ER. Conservation and accessibility of an inner core lipopolysaccharide epitope of *Neisseria meningitidis*. Infection and Immunity. 1999. 67, 5417-5426. 3. Jennings MP, Srikhanta YN, Moxon ER, Kramer M, Poolman JT, Kuipers B and van der Ley.. The genetic basis of the phase variation repertoire of lipopolysaccharide immunotypes in *Neisseria meningitidis*. Microbiology. 1999. 145, 3013-3021. 4. Mackinnon FG, Cox AD, Plested JS, Tang CM, Makepeace K, Coull PA, Wright JC, Chalmers R, Hood DW, Richards JC and Moxon ER.. Identification of a gene (*lpt-3*) required for the addition of phosphoethanolamine to the lipopolysaccharide inner core of *Neisseria meningitidis* and its role in mediating susceptibility to bactericidal killing and opsonophagocytosis. Mol Microbiol. 2002. 43, 931-43. 5. Plested JS, Harris SL, Granoff DM, Wright JC, Coull PA, Makepeace K, Gidney MA, Richards JC and Moxon ER. Highly conserved *Neisseria meningitidis* inner core lipopolysaccharide monoclonal antibody (MAb L3B5) confers protection against experimental infection. J. Infect Dis. 2003. *In press.* 6. Plested JS, Ferry BL,Coull PA, Makepeace K, Lehmann AK, Mackinnon FG, Griffiths HG, Herbert MA , Richards JC and Moxon ER. Functional opsonic activity of human serum antibodies to inner core lipopolysaccharide (*galE*) of serogroup B meningococci measured by flow cytometry. Infection and Immunity. 2001. 69, 3203-3213.

IV

In Vivo Cell Interaction and Trafficking
(Ronald Germain, Chair)

Marc Jenkins	**Imaging Cell Interactions in Lymph Nodes**
Ronald Germain	**In Vivo Analysis of the Activation of CD4 T Cells**
Ulrich Von Andrian	**In Vivo Analysis of T Cell Migration To and Within Lymph Nodes**
Christopher Contag	**Tracking Immune Cells in Cancer and Autoimmunity**

In Vivo Analysis of T Cell Migration To and Within Lymph Nodes

Ulrich H. von Andrian, M.D., Ph.D., Center for Blood Research, Harvard Medical School, Boston, MA

T cells have the seemingly impossible task of protecting every tissue in the body from every conceivable pathogen. To achieve this, T cells must migrate to numerous tissues and interact with many other cells. Naïve T cells search for pathogen-derived antigen (Ag) by constant recirculation between blood and organized lymphoid tissues, including lymph nodes (LN) and Peyer's patches (PP). Here they must interact with Ag presenting dendritic cells (DC). DC that collect antigenic material in peripheral tissues carry Ag to LN via afferent lymph vessels, whereas DC that reside in PP process Ag from the intestinal lumen. The dynamics of DC-T cell interactions in the parenchyma of these lymphoid tissues are still largely unknown. To address this question, we have developed a new intravital microscopy model that makes use of multi-photon excitation to study cellular dynamics in the three-dimensional space of LN. Our observations in this new model will be reviewed. T cell stimulation in LN and PP not only results in clonal expansion followed by assumption of effector functions, but also in tissue specific imprinting of effector homing capacity. We provide evidence that the instructions that confer migratory specificity to gut-homing effector T cells are also provided by DC; ex vivo stimulation of naive TCR transgenic $CD8^+$ cells by Ag-pulsed DC isolated from PP, but not from LN or spleen, induced the ability to home to the small intestine. In contrast, activation markers and effector functions were equivalently induced by DC from all lymphoid organs. Thus, DC not only determine whether and how naive T cells respond to a cognate Ag, but they can also confer tissue specificity to effector/memory cells to reach the anatomic site that is most likely to contain that Ag.

High resolution 4D imaging of immune cell interactions, protein distribution, and signaling in lymphoid tissues

Sabine Stoll*, Alex Huang*, Ina Ifrim*, Jérôme Delon*, Clemens Scheinecker*, Flora Castellino*, Katrin Eichelberg*, Tilmann Brotz#, Owen Schwarz+, and Ronald N. Germain*

*Lymphocyte Biology Section, Laboratory of Immunology, NIAID; +Confocal Imaging Facility, RTB, NIAID; #Experimental Immunology Branch, NCI

Immune responses involve multiple cell types often acting contemporaneously and sequential with one another and with infectious agents or antigen-bearing tissues. An important aspect of adaptive immunity involves the initiation of antigen (Ag)-specific T cell responses following recognition of peptide/MHC ligands presented by dendritic cells (DC) within secondary lymphoid tissues. Most studies characterizing these interactions involve analysis in vitro in either dispersed liquid culture or collagen gel matrices. To gain a better understanding of how T cells behave in the physiologic environment in which real immune responses take place, we have developed a method for the direct visualization of the interaction between Ag-specific T cells and Ag-bearing DC in an intact lymph node in real time. Following adoptive transfer, we used LSCM or multiphoton microscopy to track the interaction of fluorescent DC with naive antigen-specific T cells that have either been dye labeled or transduced with cDNA encoding fluorescent protein chimeras.

Using the ex vivo method we have developed, high resolution imaging of these labeled cells in explanted lymph nodes is possible for 12-15 hrs. Visualization of the initial events of an immune response in intact lymphoid tissue demonstrated that although there are a few T cells that establish only transient contacts with each of several DC, the vast majority of T cells show monogamous adherence to a single antigen bearing DC throughout the full observation period, eventually resulting in activation measurable after lymph node disruption as upregulation of CD69 on the T cells. This prolonged contact is paralleled by the formation of an immunological synapse, visualized as the exclusion of CD43-GFP from the region of membrane contact between the T cell and antigen bearing DC. After approximately 36 hrs. of contact, the T cells show clear signs of activation morphologically and detach from the antigen-bearing DC, sometimes in conjunction with cell division. The limited movement of naïve cells observed in initial confocal imaging studies seems most related to a region of low motility in the subcapsular area imaged under these conditions as compared to multiphoton studies that show substantial locomotion of naïve T cells in the absence of antigen.

These data support the view that T cell activation largely follows from prolonged association of lymphocytes with individual antigen-bearing DC rather than from the summation of signals from many brief encounters with such presenting cells. They also reveal the power of this imaging method in advancing our understanding of T cell behavior in a physiological setting. Experiments are in progress to examine early effector T cell development as determined by cytokine gene activation, to introduce FRET-based methods for following the movement and direct interaction of multiple membrane proteins or signaling molecules during the observation period, and to explore whether T

cells of the same specificity or of the CD4 and CD8 lineages can interact with a single presenting cell at the same time. Additional studies will examine the function of already mature effector T cells at peripheral sites of antigen, such as tumor beds, as well as the movement of infectious agents in epithelial and lymphoid sites, the sites of antigen processing and presentation, the interactions of B and T lymphocytes, and the activation and localization of other populations of hematopoietic effector cells such as NK and NKT cells. These studies should provide a more accurate picture of the molecular, cellular, spatial, and temporal aspects of the cell interaction and signaling events involved in host responses.

Two Distinct Dendritic Cell Populations Sequentially Present a Subcutaneous Antigen to CD4 T Cells and Stimulate Different Aspects of Cell-Mediated Immunity.

Andrea A. Itano and Marc K. Jenkins
Department of Microbiology, Center for Immunology, University of Minnesota Medical School, Minneapolis, MN 55455

Peptide:MHC II complexes derived from a fluorescent antigen were detected in vivo to identify the cells that present subcutaneously injected antigen to CD4 T cells. Skin-derived dendritic cells (DCs) that acquired the antigen while in the draining lymph nodes were the first cells to display peptide:MHC II complexes and interact with antigen-specific naive CD4 T cells. Presentation by these cells induced CD69, IL-2 production, and maximal proliferation by the T cells. Later, DCs displaying peptide:MHC II complexes migrated from the injection site via a G-protein-dependent mechanism and interacted with the T cells. Presentation by these migrants sustained expression of the IL-2 receptor and promoted delayed type hypersensitivity. Therefore, presentation of peptide:MHC II complexes derived from a subcutaneous antigen occurs in two temporally distinct waves with different functional consequences.

V

Vaccine Against Allergy and Asthma
(Dale Umetsu, Chair)

Barry Kay	**Cat Peptide Vaccines for Allergy**
Robert Coffman	**CpG Conjugate Vaccines for Allergy**
Juan Lafaille	**Regulatory Cells and IgE Synthesis**
Dale Umetsu	**Mucosal Allergan: Role of DCs, Regulatory T Cells and NKT Cells**

Ninth National Symposium on Basic Aspects of Vaccines
30 April-2 May 2003, Bethesda

A cat allergy vaccine using short overlapping T cell peptides from Fel d 1

A.B. Kay, W.L.G. Oldfield, M. Larché
Department of Allergy and Clinical Immunology, Imperial College London, National Heart & Lung Institute, London SW3 6LY, UK

Unpredictable anaphylactic reactions limit the usefulness of conventional, whole-allergen specific immunotherapy (SIT) for the treatment of atopic allergic disease. Potentially this problem can be overcome by the use of multiple, short, overlapping, peptides which avoid IgE crosslinking. The administration of peptides derived from chain 1 and 2 of the major cat allergen (Fel d 1) to cat allergic subjects inhibits the whole allergen-induced late-phase cutaneous reaction (LPCR), an established surrogate marker of allergic inflammation. We have studied the cytokine profile of cells infiltrating the LPSR before and after peptide therapy. In previous studies SIT was shown to decrease IL-4, IL-5 mRNA and protein and increase IFN-γ and IL-12 suggesting that this form of treatment produces a "switch" from a Th2 to a Th1 cytokine profile. An unexpected and at the time unexplained finding, was an increase, after SIT, in the numbers of $CD25^+$ cells at the site of cutaneous whole allergen challenge.

Recently, regulatory T cells with variable patterns of cytokine secretion have been characterized in a number of murine and human disease models. Such cells frequently express the $CD4^+/CD25^+$ phenotype but fail to proliferate *in vitro* following exposure to antigen. The mechanisms through which $CD4^+/CD25^+$ T cells and other cell types exert their immunoregulatory effects remain incompletely defined. The action of many regulatory cells is dependent upon cell-cell interactions. Expression of enhanced surface levels of the costimulation molecule CTLA-4 has been reported to be a characteristic of regulatory cells in certain models. Soluble mediators such as IL-10 and TGFβ have been shown to exert immunosuppressive effects *in vitro* and *in vivo*. Increased in IL-10 expression in blood cells has been shown after whole bee venom immunotherapy and treatment with Fel d 1-derived peptides. There were also increases in the expression of IL-10 protein and mRNA in allergen-challenged skin biopsies following immunotherapy with wasp venom suggesting that this procedure may be associated with the expansion of putative regulatory cells.

For all these reasons we have hypothesized that peptide immunotherapy is associated with the induction of immune regulation associated with $CD4^+/CD25^+$ cells expressing Th1 and/or regulatory cytokines. Fel d 1-derived peptides were administered intradermally to eight cat-allergic asthmatics. Skin biopsies were taken from whole cat allergen and diluent injected sites before and after treatment and studied by immunohistochemistry and *in situ* hybridization. The treatment was clinically effective as shown by decreases in airway hyperresponsiveness ($p=0.02$) and inhibition of the late-phase cutaneous reaction (LPCR) to allergen ($p=0.03$). Following peptide administration there was elevation (post- *vs.* pre-treatment) in the number of cutaneous $CD4^+/CD25^+$ cells, $CD4^+/IFN\gamma^+$ and cells expressing mRNA for TGFβ. These results indicate that peptide-mediated allergen hyporesponsiveness in man is associated with complex immunological alterations compatible with both the induction of tolerance and the reprogramming of Th immunity.

References

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Oldfield WLG, Kay AB, Larché M. Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. J Immunol 2001;167:1734-1739.

Oldfield WLG, Larché M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. Lancet 2002;360:47-53.

Mucosal Allergen: Role of DCs, Regulatory T Cells and NKT Cells

Dale T. Umetsu, MD, PhD
Stanford University

Administration of allergen to mucosal surfaces provokes allergic reactions in some instances, but may also induce immunological tolerance in other situations. The mechanisms by which these responses occur and by which they are regulated are unclear. We show that both oral administration of allergen in humans, and respiratory administration of allergen in mice, induce immunological tolerance, as defined by reduced proliferation of T cells to allergen. Dendritic cells (DCs) at these sites acquire the administered allergen, produce IL-10 and induce the development of regulatory T (T_R) cells that produce IL-10. Adoptive transfer of either DCs from tolerized mice or of T_R cells result in antigen specific "tolerance" in recipients. Both the development of respiratory tolerance and the function of these T_R cells require costimuation through the ICOS-ICOSL pathway.

In contrast to the induction of mucosal tolerance, administration of respiratory allergen in allergen-sensitized mice induces airway hyperreactivity (AHR), a cardinal feature of asthma. The induction of AHR in the respiratory tract requires the presence of NKT cells, since AHR fails to develop in NKT cell deficient mice. These results indicate that the cellular and molecular processes that regulate the development of tolerance versus allergic asthma are complex. However, the development of mucosal vaccines to treat allergic disease and asthma and favorably manipulate allergic immune responses requires that we fully understand these immune mechanisms.

FEATURED SPEAKER

Rafi Ahmed
Emory Vaccine Center
Emory School of Medicine

Immunological Memory: Remembering Our Pathogens

Immunological Memory: Remembering Our Pathogens

R. Ahmed, Emory Vaccine Center and Department of Microbiology & Immunology, Emory School of Medicine, Atlanta, GA 30322

Acute viral infections induce long-term humoral and cellular immunity. However, the nature of T- and B-cell memory is different. Antiviral B-cell memory is usually manifested by continuous antibody production that lasts for many years after infection or vaccination. In contrast, the effector phase of the T cell response is short-lived (a few weeks), and "memory" in the T-cell compartment results from the presence of memory T cells, which are found at higher frequencies and can respond faster and develop into effector cells (i.e., CTL or cytokine producers) more efficiently than can naïve T cells. In this talk I will discuss the following aspects of immunological memory: (1) The importance of long-lived plasma cells in maintaining humoral immunity; (2) Functional differences between naïve and memory T cells; (3) Memory T cell differentiation and memory cell subsets; and (4) Protective immunity by memory CD8 T cells.

I

Interface Between Innate and Adaptive Immunity

A1 **Targeting of Cla-1 by Synthetic Amphipathic α-Helical Containing Peptides Blocks Uptake and Proinflammatory Cytokine Response induced by bacterial component in human cells.**
Alexander V. Bocharov, CC, NIH Irina N. Baranova, CC, NIH, Tatyana G. Vishnyakova, NHLBI, Alan T. Remaley, NHLBI, Gyorgy Csako, CC, NIH, Fairwell Thomas, NHLBI, Amy P. Patterson, NHLBI, Thomas L. Eggerman, CC, NIH.

A2 **Binding and Internalization of Lipopolysaccharide by Cla-1, A Human Orthologue of Rodent Scavenger Receptor B1**
Tatyana G. Vishnyakova, NHLBI, Alexander V. Bocharov, CC, NIH, Irina N. Baranova, CC, NIH, Zhigang Chen, NHLBI, Alan T. Remaley, NHLBI, Gyorgy Csako, CC, NIH, Thomas L. Eggerman, CC, NIH, Amy P. Patterson, NHLBI

A3 **The Role of SOCS1 in T Cell Development and Activation**
By Ian M. Catlett and Stephen Hedrick
University of California, San Diego CA 92093-0687
Division of Biology & UCSD Cancer Center

A4 **Effect of Bovine Viral Diarrhea Virus on Bovine Macrophages Inflammatory Functions and Surface Marker Expression *In-Vitro***
CCL Chase, G Elmowalid, and LJ Braun, Dept. Vet Sci, South Dakota State University, Brookings, SD.

A5 **Targeting Tumor Antigens to the Mannose Receptor (CD206) on Human Dendritic Cells Leads to Colocalization with the MHC Class I Presentation Pathway and Efficient Cross-priming of CD8+ T cells.**
John E. Connolly†, Venky Ramakrishna, John F. Treml*, M. Endres*, L.Z. He*, Michael W. Fanger†, Tibor Keler* and Paul K. Wallace†*
*†Department of Immunology and Microbiology, Dartmouth Medical School, Lebanon, NH 03756, *Medarex, Inc., Bloomsbury, NJ 08804*

A6 **Eosinophil-derived neurotoxin induces dendritic cell maturation and production of a wide spectrum of cytokines**
D. Yang¶, Q. Fu, Q. Chen‡, D.L. Newton†, H.F. Rosenberg§, V. Tchernev*, M. Wang*, Z. Wang¶, E. Satyaraj*, B. Schweitzer*, S.F.*

Kingsmore, S.M. Rybak#, J.J. Oppenheim‡, D.D. Patel*, and O.M.Z. Howard‡*
*¶BRP, †ADRSP, SAIC-Frederick, #DTP, ‡LMI, NCI at Frederick, Frederick, MD 21702; §EPS, LHD, NIAID, NIH, Bethesda, MD 20892; *Molecular Staging Inc., New Haven, CT 06511.*

A7 **T_H2 and T_C2 effector T cells are not the sole determinants of Respiratory Syncytial Virus-induced eosinophilia**
S.E. Mertz, N. Gitiban, R.K. Durbin and J.E. Durbin
Children's Research Institute and Department of Pediatrics, The Ohio State University, Columbus, OH 43205, USA

A8 **Enhanced Immunogenicity to HIV-1 Envelope Using DNA Vaccines**
J.F. Bower[1], J. Sodroski[2], and T. Ross[1]. East Carolina University, School of Medicine, Greenville, NC, USA[1] and Dana Farber Cancer Institute, Boston, MA, USA.[2]

A9 **Identification of (Possible) New Class of Pattern recognition Receptors That Bind Single Base Oligodeoxynucleotides.**
Harjeet Kaur, Liliana Jaso-Friedmann, and Donald L. Evans.
University of Georgia, Athens, GA. 30306.

A10 **Elicitation of Immune Responses by a DNA Vaccine Expressing a Human Immunodeficiency Virus-Like Particle.**
Kelly R. Young[1], James A. Smith[2], Harriet L. Robinson[2], and Ted M. Ross[1]
[1]East Carolina University School of Medicine, Department of Microbiology & Immunology, Greenville, North Carolina 27858, USA and [2]Vaccine Research Center and Yerkes Regional Primate Research Center of Emory University, Atlanta, Georgia 30329, USA.

A11 **Immune-modulatory function of CG sequence motifs in covalently-closed, double-stem-loop DNA constructs**
N. Hagner, J. Köck, J. Alfken, B. Wittig, M. Schmidt.
Mologen GmbH, Berlin, Germany; Department for Molecular Biology and Bioinformatics, Fachbereich Humanmedizin, Freie Universität Berlin, Germany.

A12 **Role of Antigen Adsorption by Aluminum Hydroxide Adjuvant in Antigen Internalization by Dendritic Cells**
[a]Garry Morefield, [b]Anna Sokolovska, [b]Harm HogenEsch, [c]J. Paul Robinson, and [a]Stanley Hem
[a]Industrial & Physical Pharmacy, [b]Veterinary Pathobiology, and [c]Basic Medical Sciences
Purdue University, West Lafayette, IN 47907

A13 **Enhancement of the immunogenicity of HIV-1 genes using codon-optimisation**

Kelly Sanders and TM Ross

A14 **Virus based nanoparticles as scaffolds for display of therapeutic biomolecules**

P. Singh, A.Schneeman, M.Manchester
The Scripps Research Institute, 10550 N.Torrey Pines Rd., La Jolla, CA 92037

A15 **CD34+HPC-derived dendritic cell subsets as cancer vaccines.**

Hideki Ueno, A. Karolina Palucka, Bernard Piqueras, Sophie Paczesny, Susan Burkeholder, Joseph W. Fay and Jacques Banchereau.
Baylor Institute for Immunology Research, Dallas, TX

A16 ***P. berghei* Sporozoite Infection Induces a Autoreactive Antibody Repertoire Bearing a T_H-1 Phenotype and Specificity Towards Both the N- and C-terminal Domains of Circumsporozoite Protein**

David B. Winter, Elke S. Bergmann-Leitner, Elizabeth H. Duncan, Evelina Angov, Jackie L. Williams, Linda Huynh, Jeffrey A. Lyon, George C. Tsokos. Walter Reed Army Institute of Research, Silver Spring, Maryland, USA

A17 **Oligosaccharide Mediated Targeting of Dendritic Cells: Modulating Peripheral Tolerance**

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A18 **The Induction of Dendritic Cell Maturation by Pertussis Toxin is Toll-Like Receptor 4 Dependent**

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A19 **Monophosphoryl Lipid A as an Adjuvant for Adenovirus Vectored Vaccines**

Lawrence S. Evans, Ryan M. Swanson, A. Gregory Spies and Kenneth M. Grabstein, Sally P. Mossman Corixa Corporation, Seattle, WA 98104, USA.

A20 **Determination of the optimal form of a DNA vaccine for antigen presentation to induce an effective humoral and cell mediated response against a B-cell lymphoma.**

Deepti Aurora, Indiana State University

A21 **Differences in Localization of Free and Liposome-Encapsulated Antigens in Macrophages and Dendritic Cells**

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A22 **T_H2 and T_C2 effector T cells are not the sole determinants of Respiratory Syncytial Virus-induced eosinophilia**

S.E. Mertz, N. Gitiban, R.K. Durbin and J.E. Durbin Children's Research Institute and Department of Pediatrics, The Ohio State University, Columbus, OH 43205, USA

A23 **Modulation and potentiation of the immune response to DNA vaccines by linear MIDGE vectors coupled to peptides**

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A24 **Analysis of the Anti-gp41 Specific Immune Response in HIV-1-Positive Sera Using a Multiplexed Antibody Detection Assay**

David Opalka[1], Antonello Pessi[2], Elisabetta Bianchi[2], William Schleif[5], Michael McElhaugh[5], Renee Danzeisen[4], Romas Geleziunas[4], Michael Miller[4], Debra Eckert[3], David Bramhill[3], Joseph Joyce[6], James Cook[6], William Magilton[6], Naren Chirmule[1], John Shiver[5], Emilio Emini[5] and Mark Esser[1] [1]Virus and Cell Biology, Merck Research Laboratories (MRL), 466 Devon Park Dr., Wayne, PA 19087-8630. [2]IRBM P. Angeletti, Via Pontina Km 30.600, Pomezia, RM, Italy. [3]Biologics Research, MRL, Rahway, NJ, 07065. [4]Biological Chemistry, [5]Viral Vaccine Research, [6]Virus and Cell Biology, MRL, West Point, PA, 19486

A25 **Indoleamine 2,3-Dioxygenase Expression is Increased in Immature Human Dendritic Cells Exposed to Melanocyte Derived gp100**

Hui Fang Dong[1], J. J. Oppenheim[2], O. M. Zack Howard[2] [1]Basic Research Program SAIC-Frederick, [2]Laboratory of Molecular Immunoregulation, CCR, NCI-Frederick, Frederick MD 21702

A26 **Coxsackievirus B3: Analysis of a Recombinant RNA Vaccine for its Protective Effect against Subsequent Virus Challenge in Mice**

Isabelle P. Hunziker, Ralph Feuer, Stephanie Harkins, and J. Lindsay Whitton

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A27 **The retinal autoantigens, Interphotoreceptor retinoid-binding protein (IRBP) and S Antigen, are chemotactic for human T lymphocytes and immature Dendritic cells**

Huifang Dong, De Yang, Saobo Su, Rachel R. Caspi, O.M. Zack Howard, and Joost J. Oppenheim

A1

Targeting of Cla-1 by Synthetic Amphipathic α-Helical Containing Peptides Blocks Uptake and Proinflammatory Cytokine Response induced by bacterial component in human cells.

Alexander V. Bocharov, CC, NIH Irina N. Baranova, CC, NIH, Tatyana G. Vishnyakova, NHLBI, Alan T. Remaley, NHLBI, Gyorgy Csako, CC, NIH, Fairwell Thomas, NHLBI, Amy P. Patterson, NHLBI, Thomas L. Eggerman, CC, NIH.

Lipopolysaccharides (LPS) as well as chaperonin 60 (Gro-EL) and lipoteichoic acid (LTA) are proinflammatory bacterial cell components implicated in the pathogenesis of sepsis, septic shock and proinflammatory diseases. Recently, we have demonstrated that Cla-1, human HDL-receptor, mediates cellular LPS-binding and internalization. Since the major recognizing motif in SR-BI ligands is a double anionic amphipathic α-helix, the purpose of this study was to evaluate the effects of Cla-1 targeting with synthetic double amphipathic α-helical containing peptides on LPS-uptake and LPS-, LTA and Gro-EL- stimulated cytokine production in HeLa and THP-1 cells. The L-37PA peptide which contains two class A amphipathic helices linked by proline efficiently competed against iodinated LPS for Cla-1. Both Alexa-L-37PA and Bodipy-LPS were found to colocalize at the cell surface and were transported by Cla-1 to a perinuclear compartment, which was identified as the Golgi complex. A 100-fold excess of L-37PA nearly eliminated Bodipy-LPS uptake in the cells, blocking both LPS-binding to the plasma membrane and LPS-internalization. The L-37PA as well as the D-37PA peptide synthesized with D-amino acids blocked LPS-stimulated cytokine secretion (IL-6, IL-8, and TNF-α) and mRNA expression in THP-1 cells. The 37PA peptide synthesized with two D-amino acid substitutions in each helix disturbing the helical structure (L2D-37PA) as well as the single helical peptide, 18PA, a mono-helical form of L-37PA, did not affect either LPS-uptake or LPS-stimulated cytokine secretion in THP-1 cells. The L-37PA and D-37PA peptides (but not 18A or L2D-37PA) also blocked both lipotecoic (LTA) acid and Gro-EL induced cytokine secretion in THP-1 cells. These results indicate that synthetic peptides, which target Cla-1, block LPS uptake and cytokine stimulation by LPS, LTA and Gro-EL indicating the double amphipathic helical motif of exchangeable apolipoproteins may represent a general host defense mechanism against inflammatory reactions. The peptides may have a potential use as therapeutic agents against infections and inflammation.

A2

Binding and Internalization of Lipopolysaccharide by Cla-1, A Human Orthologue of Rodent Scavenger Receptor B1

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Lipopolysaccharides (LPS) are highly glycosylated anionic phospholipids contributing to septic shock and demonstrate significant structural similarities to anionic phospholipids. Human HDL-receptor, Cla-1, mediates binding of HDL, low-density lipoprotein (LDL), exchangeable apolipoproteins and negatively charged phospholipids. These data demonstrate that Cla-1 also functions a lipopolysaccharide (LPS)-binding protein and endocytic receptor mediating the binding and internalization of lipoprotein-free, monomerized LPS as well as lipoprotein-associated LPS. Stably transfected HeLa cells expressing Cla-1 bound LPS with a Kd of about 16 μg/ml and had a 4-fold increase in binding capacity and LPS uptake. Bodipy-labeled LPS uptake through Cla-1 was found to initially accumulate in the plasma membrane and subsequently in a peri-nuclear region identified predominantly as the Golgi complex. When associated with HDL, LPS uptake was also increased in Cla-1 overexpressing HeLa cells by 5-10 fold. The rate of Cla-1 mediated [^{3}H] LPS uptake exceeded [^{125}I] apolipoprotein uptake by 5-fold indicating a selective LPS uptake. Upon interacting with Cla-1 overexpressing HeLa cells, the complex (Bodipy-LPS/Alexa 488 apolipoprotein labeled HDL) bound and was internalized as a holoparticle. Intracellularly, LPS and apolipoproteins were sorted to different intracellular compartments. With LPS associated HDL, intracellular LPS colocalized predominantly with transferrin, indicating delivery to an endocytic recycling compartment. In summary, Cla-1 was found to bind and internalize both monomerized and HDL-associated LPS transporting LPS to two distinct compartments where one (the Golgi complex) is a predominant location of signaling for Toll Like receptors while another is involved with molecular sorting or bile excretion. These data indicate that Cla-1 may play important role in LPS-induced inflammation as well as taking part in hepatic LPS-uptake, degradation and excretion.

A3

The Role of SOCS1 in T Cell Development and Activation

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Suppressor of Cytokine Signaling 1 (SOCS1) is a critical regulator of T cell development and activation. SOCS1 is a negative feedback inhibitor of Janus kinases. Study of SOCS1-/- T cells has been hampered by their spontaneous activation and the premature death of the animal. A T cell receptor transgene specific for ovalbumin (OTI) prolongs life. This tg partially rescues the activated phenotype of SOCS1-/- T cells; OTI+ SOCS1-/- T cells have a central memory or homeostatic proliferation phenotype: $CD25^{neg}$, $CD69^{neg}$, $CD44^{hi}$, $CD62L^{hi}$, $Ly6C^{hi}$ and elevated CD122 (IL-2 receptor β). However, thymic $CD4^{-}CD8^{+}$ single positive cells have a naïve phenotype: $CD44^{lo}$ $CD69^{neg}$, $CD25^{neg}$. OTI+ SOCS1-/- thymic single positive and peripheral T cells are hyper-proliferative to IL-2 and IL-15 alone as well as TCR stimulation by antigen or anti-CD3. They also secrete more IL-2 and IFNγ in response to TCR ligation. Thus, SOCS1 may regulate the responsiveness of T cells to antigen receptor signaling. Rag^{0} OTI+ SOCS1-/- mice do not have the activated T cell phenotype but retain the hyper-activity and appear healthy. Thus, the activated phenotype requires endogenous Vα rearrangements. The lethal phenotype is likely the result of both failure to control autoreactive T cells as well as the failure to control the resulting cytokine production. Potentially, loss of SOCS1 may affect T cell development, control of naturally occurring autoimmune cells or the responses of non-autoreactive T cells. Current data suggest that all three are possible and are under investigation.

A4

Effect of Bovine Viral Diarrhea Virus on Bovine Macrophages Inflammatory Functions and Surface Marker Expression *In-Vitro*. CCL Chase, G Elmowalid, and LJ Braun, Dept. Vet Sci, South Dakota State University, Brookings, SD.

Macrophages play a central role in the defense against intracellular pathogens; and are keystones in innate immune response and in development of acquired immune response against viral infection. Bovine viral diarrhea virus (BVDV) is a member of the flavivirus family, a family that also includes Dengue Fever virus and hepatitis C virus. BVDV is a devastating cattle pathogen. BVDV isolates are divided into cytopathic (cp) and noncytopathic (ncp) biotypes according to their effect on bovine cell culture *in vitro*. Infection of cattle with BVDV is usually associated with mild, acute or severe acute diseases; persistent infection; immunosuppression; and secondary microbial infection depending on BVDV strain virulence. The involvement of macrophages in BVDV induced immunosuppression is still need investigation and differences in virulence among BVDV strains on macrophage inflammatory function are still unclear. In this study, *in vitro* effects of eight BVDV strains of different virulence isolated from persistently or acutely diseased animals on monocytes-derived macrophage (MDM) inflammatory functions and five surface markers were investigated. MDM were found to be susceptible to both BVDV biotypes. Cp BVDV strains induced morphological changes and down-regulated nitric oxide (NO) production upon stimulation with lipopolysaccharide (LPS), while ncp BVDV strains did induce up-regulation of NO production upon stimulation with LPS, but no morphological changes. Virulent (cp or ncp), but not persistent BVDV strains impaired MDM phagocytic capacity, bactericidal and fungicidal activities and down-regulated NO production post infection (p.i) with live *Escherichia coli* and /or *Candida albicans*. The effect on MDM inflammatory functions and MHC I, MHC II, CD14, CD11b and CD11c expression was varied among the eight VBDV strains Cp BVDV up-regulated MHC I, while ncp down-regulated MHC I. Both MHC II and CD14 expression level were significantly down-regulated, while CD11b and CD11c expression level did not change following infection with either virulent or persistent strains. Inactivated BVDV neither induced cell pathology nor functional changes. These results demonstrated that replicating BVDV virulent strains infection can reduce macrophages inflammatory functions and surface marker expression. This reduction may contribute to the immunosuppression, and the secondary microbial infection associated with BVDV infection and the difference in the disease severity in cattle infected with BVDV. Studies are currently underway to determine the effect of BVDV infection of antigen presentation cells on antigen specific lymphocyte proliferation.

Targeting Tumor Antigens to the Mannose Receptor (CD206) on Human Dendritic Cells Leads to Colocalization with the MHC Class I Presentation Pathway and Efficient Cross-priming of CD8+ T cells.

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Abstract

The multi-lectin pattern recognition receptor mannose receptor (CD206) is an important component of dendritic cell antigen uptake machinery. The purpose of this study was to elucidate the pathway of antigen uptake by CD206 in immature human monocyte-derived dendritic cells (MO-DC) and to evaluate its efficacy as a targeting molecule for tumor immunotherapy. Confocal microscopy, using a human antibody (B11) specific for the mannose receptor, demonstrated that the CD206 is rapidly internalized and remain internal at 3 hours in MO-DCs. This internalization was clathrin dependant and differed significantly from mannosylated antigen uptake which appeared to be primarily through nonspecific fluid phase mechanisms. Antibodies directed against CD206 did not co-localize with late endocytic and lysosomal markers RAB7 and LAMP-1, nor was there any co-localization with Class II containing compartments. Internalized CD206 however, did demonstrate significant co-localization with Class I containing vesicles both at the periphery and in internal vesicular compartments. This co-localization was followed by an apparent sorting of Class I from CD206 containing vesicles. These observations are consistent with data demonstrating a functional interaction between dendritic cell CD206 and Class I antigen presentation in the generation of anti-tumor CTL responses. A chimeric targeting construct consisting of the heavy chain of the B11 antibody fused to the tumor antigen pmel-17, was able to efficiently elicited class I restricted T cell responses to antigen pulsed targets as well as cell lines expressing the pmel-17 antigen. Due to its restricted expression, efficiency of antigen capture, intracellular association with Class I containing vesicles, and ability to generate an effective cross-priming response to tumor antigens, CD206 is an attractive targeting molecule for anti-tumor immunotherapy.

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Dendritic cells, Antigen Presentation/Processing, Mannose receptor, CD206

Eosinophil-derived neurotoxin induces dendritic cell maturation and production of a wide spectrum of cytokines

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Most mammalian antimicrobial proteins generated by neutrophils and epithelial cells have chemotactic and activating effects on host cells, particularly, cells of the host immune system. Eosinophil granules also contain antimicrobial proteins including eosinophil-derived neurotoxin (EDN), a protein belonging to the ribonuclease (RNase) A superfamily. EDN has recently been found to have antiviral and chemotactic activities *in vitro*. Here we report that EDN can induce human dendritic and monocytic cells *in vitro* to produce a variety of cytokines, chemokines, growth factors, and soluble receptors. Most of the mediators that were induced by EDN were proinflammatory, such as IL-6, TNFα, ENA-78, IP-10, MIP-1α, MIP-1β, MCP-1, MCP-2, MCP-3, and Rantes. Although not having been shown to have antimicrobial activity, human pancreatic ribonuclease (hPR), another member of the ribonuclease (RNase) A superfamily, demonstrated similar activities. Furthermore, EDN and hPR also possess the capacity to induce the maturation of dendritic cells as evidenced by upregulation of CD83, CD86, HLA-DR, and CCR7 expression. In contrast, human angiogenin, a RNase A superfamily member that is evolutionarily more distant from EDN and hPR did not induce cytokine production. Thus, both EDN and hPR can act as potent activators for human dendritic and monocytic cells and may be involved in various host defense and/or inflammatory processes.

T_H2 and T_C2 effector T cells are not the sole determinants of Respiratory Syncytial Virus-induced eosinophilia

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Respiratory Syncytial Virus (RSV) is an important pediatric pathogen and a major cause of lower airway disease in infants and young children. Despite the magnitude of the clinical problem, no safe and effective vaccine is yet available. This is due in part to the difficulties inherent in immunizing young infants, but work has also been inhibited by the legacy of vaccine-enhanced disease seen following trials of a formalin-inactivated, alum precipitated RSV vaccine preparation that took place in the 1960s. Enhanced disease was eosinophilic in character and associated with a T_H2 cytokine response to viral antigen. We have observed that mice lacking the interferon (IFN) signal transducer STAT1 suffer from enhanced eosinophilic RSV disease following primary infection in the absence of priming.

In infected 129 SvEv STAT1-/- mice, severe eosinophilic lung pathology is associated with IL-5 and IL-13 production from RSV-F specific $CD4^+$ and $CD8^+$ T cells. In light of the important role that IFN-γ and its downstream mediator STAT1 are known to play in development of the T_H1 response, this is not unexpected. To determine whether these effects were entirely due to the lack of IFN-γ effects, we infected a series of 129SvEv mouse strains lacking the IFN-α receptor (IFN-α/β R-/-), the IFN-γ receptor (IFN-γ R-/-), both IFN receptor chains (IFN-$\alpha/\beta/\gamma$R-/-), or STAT1. We found pronounced eosinophilic lung disease in mice lacking both IFN receptors or STAT1, but only small numbers of eosinophils in the lungs and BAL fluids of infected IFN-γ R-/- animals. These significant differences in pathology occurred despite the fact that all three of these strains developed T_H2 or T_C2 cytokine responses to RSV-F protein epitopes that elicited T_H1 or T_C1 cytokines from immune wild type or IFN-α/β R-/- splenocytes. We conclude from these studies that although CD4+ T_H2 cell differentiation will occur in the absence of IFN-γ, the presence of an intact IFN-α/β signaling pathway inhibits the development of eosinophilic disease. Supported by NIH grant AI47226 to JED.

Enhanced Immunogenicity to HIV-1 Envelope Using DNA Vaccines

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DNA vaccines expressing the envelope (Env) of the human immunodeficiency virus (HIV) have been relatively ineffective at generating high titer, long-lasting immune responses. Oligomeric/trimeric forms of Env that more closely mimic the native proteins on the virion are often more effective immunogens that monomeric Envs. In this study, several forms of Env (YU-2) were tested for their immunogenic potential: two soluble, trimeric forms of Env ($sgp140_{YU-2}$) stabilized with a synthetic trimer motif were compared to $sgp140_{YU-2}$ without any synthetic trimerization domain, as well as to monomeric gp120. DNA plasmids were constructed to express Env alone or fused to various copies of murine C3d (mC3d). BALB/c mice were vaccinated (day 1 and week 4) with DNA expressing a codon optimized envelope gene inserts alone or fused to mC3d. Mice were subsequently boosted (week 8) with the DNA or recombinant Env protein. All mice had high anti-Env antibody titers regardless of the use of mC3d. Sera from mice vaccinated with DNA expressing non-C3d fused timers and then boosted with recombinant Env were able to neutralize viral infection *in vitro* at a 1:20 dilution. In contrast, only mice inoculated with DNA expressing Env-C3d proteins had the highest neutralizing titers (1:40). The results of this study show that DNA priming using codon optimized DNA vaccines expressing various Envs followed by either DNA or protein boost, elicit similar, high levels of anti-Env antibodies. However, only mice vaccinated with DNA expressing trimeric forms of Env coupled to C3d had enhanced neutralizing antibody titer.

Identification of (Possible) New Class of Pattern recognition Receptors That Bind Single Base Oligodeoxynucleotides.

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Abstract

Innate immune cells recognize pathogens by detecting pathogen-associated molecular patterns (PAMP) that are distinct from those of the host. One such pattern is unmethylated CpG dinucleotides which are common in bacterial DNA but not in vertebrate genomes. Previously it was shown by others that synthetic oligodeoxynucleotides (ODNs) containing CpG-motifs activate natural killer cells. Interstingly, poly-deoxyguanosine(dG) and dG-containing ODNs in the absence of CpG motifs are shown to be mitogenic for B cells, macrophages and costimulate $CD8^+$ T cells. The present study was designed to examine the effects of 20 mer of single base oligodeoxyguanosine (dG20) on nonspecific cytotoxic cells (NCC) of teleost and to identify the membrane proteins on NCC and mammalian cells that bind single base oligodeoxynucleotide (ODN) ligands. ODN dG20 was first examined for binding to NCC and to (mouse) RAW 264.7 and (human) THP-1 cells. Binding to NCC by dG20 was specific and saturable at 0.06 μg/5 x 10^4 cells. Saturable binding to RAW and THP-1 cells by dG20 occurred at 0.02 μg/5 x 10^4 cells and 0.08 μg/ 5 x 10^4 cells, respectively. Southwestern blots of whole cell lysates and immunoprecipitation of biotin labeled cell membranes from NCC, RAW 264.7 and THP-1 cells demonstrated two different mw species (14-18 kDa and 29-34 kDa) of binding proteins. These were crossreactive by Western blot examination with a polyclonal anti-histone-1 antibody. Activation studies revealed that dG20 activated a 2-fold upregulation of membrane binding of homologous dG20-biotin. dG20 also stimulated NCC increased membrane expression of NCCRP-1. The single base ODN binding proteins may represent a new class of pattern recognition receptors that are involved in innate anti-bacterial resistance mediated by NCC.

A10

Elicitation of Immune Responses by a DNA Vaccine Expressing a Human Immunodeficiency Virus-Like Particle.

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The proposed vaccine has been designed to afford the broadest possible response in vaccinated individuals with the goal of generating multi-epitope responses. The DNA immunogen expresses the Gag, Pol, Env, Tat, Rev, Vpu proteins of HIV-1 and produces a noninfectious virus-like particle (VLP) as determined by sucrose density gradient ultracentrifugation. Mutations have been introduced into the VLPs to severely restrict packaging of viral RNA and reverse transcription activity, as well as the inhibition of cellular integration. However, these VLPs vaccines should bind to appropriate cell surface receptors and infect susceptible cells. Envelopes from different R5-tropic (B clades) strains of HIV-1 were cloned into the Gag-Pol (clade A-G) DNA backbone in order to induce high titer, cross-reactive anti-Env antibodies. Each VLP elicited efficient immune responses against multiple gene products. This research was supported by grants NIH AI-49061 & NIH AI-51213 (T.M.R.).

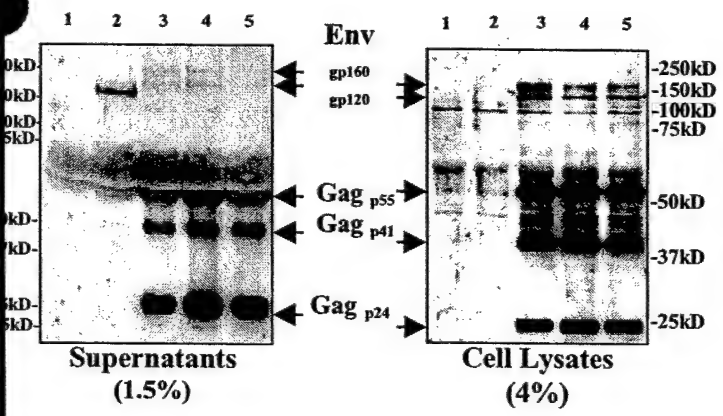

Figure A: In vitro expression of virus-like particle vaccines. Cell lysates and supernatants of 293T (human embryonic kidney) cells transiently transfected with vaccine plasmids were electrophoresed through a SDS-PAG and analyzed by western hybridization using HIV-Ig. Lane 1 (vector), lane 2 (secreted ADA Env gp120), lane 3 (ADA-VLP), lane 4 (89.6-VLP) and lane (R2-VLP).

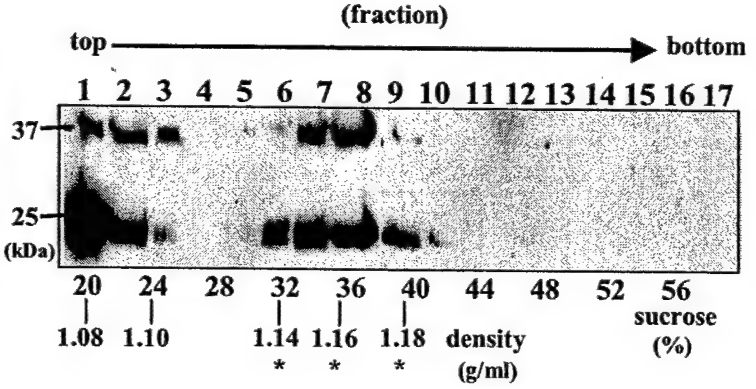

Figure B: Particle purification by sucrose density gradient. Cell supernatants of COS (monkey fibroblasts) cells transiently transfected with 89.6-VLP DNA were harvested, pelleted through a 20% sucrose cushion, and overlaid onto a 20-60% sucrose density gradient for 17 hours at (24,000rpm-SW40Ti). Fractions (17, 750ul each) were collected from top to bottom of the gradient. The proteins were analyzed by western hybridization using HIV-Ig. The viral proteins (p41 and p24) detected in fractions 6-9 represent proteins incorporated into particles (*), while viral proteins found in fractions 1-3 are not incorporated into a particle.

Immune-modulatory function of CG sequence motifs in covalently-closed, double-stem-loop DNA constructs

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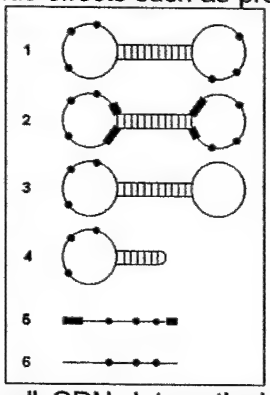
Oligonucleotides (ODN) with unmethylated CG sequence motifs are potent modulators of the immune response via activation of lymphocytes, NK-cells and professional APCs, cytokine production (i.e. IL-2, IFN-γ, IL-12p40, IL-6), and expression of surface markers (i.e. CD80/ B7.1, CD40, MHC-II). They have demonstrated a wide utility as therapeutic agents, i.e. as vaccine adjuvants for cancer gene therapy and as immunostimulators for the treatment of allergic diseases. Generally, phosphorothioate (PS) backbones were used for exonuclease protection leading also to some non-specific side effects such as prolongation of coagulation time. Here, we analyzed the stimulatory potential of a new class of molecules, double-stem-loop immunomodulators (dSLIM) with phosphorodiester (PO) backbones and a dumbbell-like covalently-closed structure (see figure): ODN-1 with CG motifs in both loops; ODN-2 with PS-residues (gray boxes); ODN-3 with CG motifs only in one loop; ODN-4 with one loop; and two controls: ODN-5, a linear PS-ODN; ODN-6, a linear PO-ODN. A significant stimulation of CD80, CD40, MHC-II and ICAM-1 presentation on B-cells was detected with all ODN. Interestingly, ODN-2 had the greatest effect, followed by ODN-1 and ODN-5. The least increase was seen with ODN-3, -4 and -6. No effect was detected in T-, NK-cells or monocytes. IL-12p40 production was significantly increased by all ODN with a greater effect of ODN-2. The increase of IL12p40 was B-cell restricted as well. Furthermore, ODN-1, -5 and -6 significantly increased IFN-γ production in $TLR9^+$, but neither $TLR9^-$ NK-cells nor other tested cells. Thus, dSLIM constitutes a new class of molecules for potential use in various applications, i.e. as vaccine adjuvant in gene therapy.



A12

Role of Antigen Adsorption by Aluminum Hydroxide Adjuvant in Antigen Internalization by Dendritic Cells

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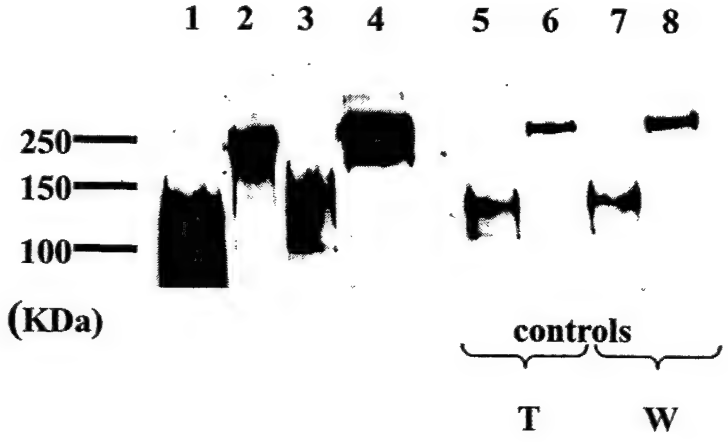
An important step in the induction of the immune response by vaccines is the internalization of antigen by antigen presenting cells such as dendritic cells (DCs). Pinocytosis and phagocytosis are two mechanisms of antigen internalization by DCs. In many vaccines the antigen is adsorbed to aluminum hydroxide adjuvant (AH). Following intramuscular or subcutaneous administration the vaccine is immersed in interstitial fluid. Upon exposure to interstitial fluid the antigen may either elute or stay adsorbed to the adjuvant surface. Antigens, which elute from the adjuvant surface must be internalized through pinocytosis by DCs while those that remain adsorbed must be internalized through phagocytosis by DCs. This study was undertaken to determine if the degree of antigen adsorption following administration affects the efficiency of antigen internalization by DCs.

Alpha-casein (AC) labeled with a green fluorescent dye was selected as the model antigen. AC has 8 phosphate groups and was adsorbed to AH through the ligand exchange mechanism of adsorption. In order to model vaccine antigens which elute from AH following administration, DCs were incubated with a solution of AC. To model vaccine antigens that do not elute from AH following administration, DCs were exposed to AC adsorbed to AH. The uptake of antigen by DCs was determined at 0.5, 1, 2, and 3 hr. by confocal microscopy and flow cytometry. DCs internalized both the AC in solution and the AC/AH complex. However, the mean fluorescence intensity of DCs incubated with adsorbed AC was 5x as high as DCs incubated with soluble AC. It was concluded that antigen adsorption to AH, following administration enhances antigen internalization by DCs.

Enhancement of the immunogenicity of HIV-1 genes using codon-optimisation

Kelly Sanders and TM Ross

DNA vaccination has been shown to be an effective strategy to elicit both humoral and cellular immune responses. In this study, we have postulated that codon optimized genes of the HIV-1 envelope in a DNA vaccine will considerably enhance both humoral and cellular immune responses. A synthetic HIV-1 gp120 sequence in which most wild-type codons are replaced with codons from highly expressed human genes (cogp120) were utilized. Each vaccines expresses wild-type (wt) gp120 or cogp120 from the HIV-1JRFL alone or fused to three tandem copies of the murine C3d. C3d is a component of the complement system and, when fused to an antigen, has been shown to be enhance the immune response to an antigen as much as 10,000 fold. Therefore, each DNA vaccine was inoculated into BALB/c mice and evaluated for the elicitation of both humoral and cell-mediated immune responses.



Expression of vaccine constructs *In Vitro.*
1.Wt JRFL 2. Wt JRFL-mC3d 3. coJRFL 4. coJRFL-mC3d
5. 89.6 6. 89.6mC3d 7. 89.6 8. 89.6-mC3d
T= Transfection W= Western

As demonstrated by the above western, *invitro,* codon optimization of JRFL envelope would appear to have no positive effect on increasing the protein expression levels compared to wild-type JRFL. This may be due to the fact that JRFL is atypical of most HIV-1 strains and appears to have sufficient humanization prior to codon optimization, thus producing no evident effect. Codon optimization of JRFL additionally coupled to C3d would appear to have increased the levels of protein expression, as expected. However, the JRFL-mC3d constructs still did not produce levels on par with those produced by the wt JRFL.

Immunology Conference

Walter Reed Army Institute of Research Apr 30-May 2nd.

Session: Interface between innate and adaptive immunity

Virus based nanoparticles as scaffolds for display of therapeutic bio-molecules

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Our primary goal is to produce inexpensive therapeutic bio-molecules. Two virus based nanoparticle platforms have been developed towards this objective: Cowpea mosaic virus (CPMV) and flock house virus (FHV), which are infectious to plants and insect cells respectively, are inert in mammals. Having known the structure of these viruses in extensive detail, display of foreign peptides (genetically and chemically) was feasible on the virus surface. Interestingly, mice were fed with these virus based nanoparticles (VBNPs) revealed that they traffic to various deep tissues before their removal from the body. The VBNPs are stable and can withstand changes in pH and wide range of temperature. These observations have prompted us to utilize these VBNPs in economical production of therapeutic peptides as well as for developing novel oral therapeutics. One of our investigations is focused on preventing diabetes type 1, an auto-immune disease, by VBNP based induction of regulatory T-cells. For this purpose an altered peptide ligand (a modified self-antigen) was displayed on CPMV. The chimera will be evaluated in a non-obese diabetic mouse model by parentral and oral administration. This study will open avenues to developing treatment regimens for other auto-immune diseases in which peripheral tolerance can be stimulated using the VBNPs displaying modified self peptides. The VBNPs will provide us in generating inexpensive vaccines and possibly edible therapeutics.

A15

CD34+HPC-derived dendritic cell subsets as cancer vaccines.

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Dendritic cells (DCs) are increasingly used in cancer therapy. Early trials in humans have shown the safety of cancer antigens-loaded DCs as well as some clinical and immune responses. However, many issues remain to be addressed including the choice of the DC subset to be administered and the way to generate it. The most popular way to generate DCs is to culture blood monocytes with GM-CSF and IL-4, which yield a uniform population of immature DC resembling interstitial DCs (intDCs). This contrast with hematopoietic stem cells that, when cultured with GM-CSF and TNF, yield preparations that include intDCs as well as Langerhans cells (LCs). While GM-CSF/IL-4 induced DCs require additional maturation factors, CD34-DCs do not as they are generated in the presence of TNF alpha, a DC activation factor. We have vaccinated 18 HLA A*0201^{+} patients with stage IV melanoma with CD34-HPC-derived DCs pulsed with six Ags: influenza matrix peptide (Flu-MP), KLH, and peptides derived from the four melanoma Ags: MART-1/Melan A, gp100, tyrosinase and MAGE-3 (Banchereau et al. Cancer Res. 2001 61:6451-6458). Analysis at 10 weeks (after 4 vaccinations) revealed an immune response to control antigens in 16/18 patients. An enhanced immune response to 1 or more melanoma antigens (MelAg) was seen in these 16 patients. The 2 patients failing to respond experienced rapid tumor progression. 6/7 patients with immunity to 2 or less MelAg had progressive disease 10 weeks after study entry, in contrast to tumor progression in only 1/10 patients with immunity to > 2 MelAg.

We are now studying why this vaccine shows such significant immune responses. An obvious reason is the presence of three DC subsets which might act in synergy to induce potent immune responses. We will present genomic and functional characteristics of these three DC subsets and of T cells which were activated by these DC subsets.

A16

P. berghei Sporozoite Infection Induces a Autoreactive Antibody Repertoire Bearing a T_H-1 Phenotype and Specificity Towards Both the N- and C-terminal Domains of Circumsporozoite Protein.

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Repeated malaria infection induces a high titer of antibodies to the sporozoite-stage antigen, Circumsporozoite Protein (CSP). The majority of these infection-induced antibodies appear to bind the repeat domain of CSP, while there are lower quantities of antibodies to the flanking N- and C-terminal regions. There is little evidence that repeated infection induces immunological memory or affinity maturation to these epitopes. This suggests that CSP, in the context of infection, induces either a purely T-independent response, or a combination of low affinity T-independent and T-dependent responses. In this study, normal Balb/c or Nu^-/Nu^- mice on a Balb/c background were infected subcutaneously with 400-4000 live *P. berghei* sporozoites and hybridoma libraries were constructed from their spleen cells 4-14 days later. The monoclonal antibodies from the hybridomas were screened first for binding to sporozoites and then for isotype expression and fine specificity. While the antibodies from the nude mice typically expressed IgM heavy chain, the late primary antibodies (day 14 post inoculation) from the normal Balb/c mice predominately expressed IgG2a. This suggests that they were stimulated in a T_H-1 dependent manner. The majority of antibodies from both strains of mice bound either to the N-terminal fragment alone or to both the N- and C-terminal fragments. Since autoantibody levels rise during malaria infection, we speculated that the hybridomas might bear autoreactive antibodies. We therefore tested the ability of this group of antibodies to bind a panel of auto-antigens (ssDNA, actin, myosin, and keratin). Most of the antibodies bound to one or more auto-antigens, but there was a difference in the binding pattern of antibodies from nude vs. normal mice. This supports the idea that T-dependent and T-independent pathways of activation can induce different repertoires of antibodies. This study is also the first to correlate the reported increase in autoantibodies during malaria infection with the cognate induction of CSP-specific antibodies.

A17

Oligosaccharide Mediated Targeting of Dendritic Cells: Modulating Peripheral Tolerance

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Recent experimental evidence lends strong support to the hypothesis that dendritic cells (DCs), the most efficient of the professional antigen presenting cells, play a crucial role in defining and maintaining immunological self in the peripheral zones of the body by deleting self-reactive T cells or stimulating the production of regulatory T cells which, in turn, act on self-reactive T cells. It is believed that in the absence of infection or inflammation, the so-called steady state, DCs constantly acquire self-proteins from dying tissue and innocuous environmental sources from the regions of the periphery they patrol. DCs presenting these captured and processed proteins are held to be tolerogenic.

The research presented here focuses on the synthesis of neoglycoprotein conjugates of "self" proteins bearing complex oligosaccharides and the use of such constructs to specifically target the dendritic cell (DC) specific C-type lectin DC-SIGN *in vivo*. Specific uptake of these targeted self-antigens by DCs without providing additional maturation signals should result in immunological tolerance. Here the immunological consequences of targeting model antigens to DCs in the steady state will be presented, as well as the mechanisms of antigen uptake and processing. Both *in vitro* tissue culture experiments and *ex vivo* flow cytometry and confocal microscopy experiments will be performed to demonstrate the DC-specific nature of the oligosaccharide mediated targeting. The use of such complex carbohydrate structures may afford an efficient means of targeting DCs with antigens associated with autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, and juvenile diabetes.

A18

The Induction of Dendritic Cell Maturation by Pertussis Toxin is Toll-Like Receptor 4 Dependent

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ABSTRACT

Pertussis toxin (PT) is a major virulence factor of *Bordetella pertussis* which can exert a range of effects on the immune system, including augmenting the activation of cell-mediated immune responses to foreign antigens (Ags). PT has been shown to be a potent activators of immunocompetent cells such as dendritic cells. However, the mechanism of PT induced adjuvanticity remains undefined. Since dendritic cells (DC) have a central role in the initiation of immune responses, we investigated the effects of maturation of dendritic cells in response to PT. We showed that PT directly induced maturation of both human and murine DC in vitro. PT- treated immature DC expressed CD83 and CD86, and produced pro-inflammatory cytokines (IL-6, TNF-α and IL-12). Most importantly, the maturation of dendritic cells exhibited the characteristics of a Toll-like receptor (TLR)-4 response. Bone marrow–derived DCs from C3H/HeJ mice with mutant TLR-4 alleles were unresponsive to PT. Conversely, DCs from C3H/HeN mice responded to PT. Furthermore, PT enhanced the luciferase activity and IL-8 production of TLR4 transfected HEK293 cells, but not those of TLR2 transfected HEK293 or nontransfected HEK 293 cells. These results indicate that the *Bordetella pertussis* derived exotoxin, PT can induce the maturation of DC and presumably promotes Th1 responses by using TLR 4 pathways. These results have identified a new type of pathogen-associated molecular pattern response for TLR4, which can account for the immunoadjuvant effect of PT.

A19

Monophosphoryl Lipid A as an Adjuvant for Adenovirus Vectored Vaccines

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Immunization with recombinant adenovirus is a potent means of eliciting a high frequency CD8+ T cell response together with robust CD4+ T cell and humoral responses. The use of adenovirus vaccines is limited however, by neutralizing antibody responses to the vector that restrict the ability to boost with the same construct. In addition, there are safety concerns associated with delivery of high doses of adenoviral particles to humans.

We have developed a formulation consisting of adenovirus admixed with an aqueous formulation of the adjuvant, Monophosphoryl Lipid A (MPL-AF). MPL was derived from the detoxified LPS of *Salmonella minnesota*, strain R595. Preliminary data now suggests that MPL, like LPS, interacts with TLR-4 to induce immediate activation of cells of the innate immune system resulting in the secretion of inflammatory cytokines, reactive oxygen intermediates and defensins. This inflammatory response in turn induces and regulates the initiation of the antigen-specific adaptive immune responses, controlling DC maturation and differentiation of T helper cells.

A Corixa proprietary *Mycobacterium tuberculosis* antigen, TbH9 (Mtb39A), was cloned into a recombinant E1 and E3 deleted, replication-defective adenovirus, serotype 5. The adenovirus vector (Ad5-TbH9) was used as a model in C57BL/6 mice to assess antigen specific immune responses induced by our formulation.

Mice immunized intradermally sub-cutaneously, or intramuscularly with Ad5-TbH9 admixed with MPL elicited enhanced CD8+ T cell responses to TbH9 relative to those induced by adenovirus alone, as measured by CTL assay, intracellular IFN-γ staining and by IFN-γ ELISPOT. CD4+ T cell responses were also enhanced as measured by interferon-gamma production by splenocytes. Interestingly, although this formulation induces an increase in the titer of ELISA antibodies to adenovirus, we did not detect an increase in antibodies that neutralize viral infectivity, as measured by in vitro assay.

In addition to enhancing the immune response generated by immunization with an optimal dose of adenovirus, MPL can be used to lower the dose of virus used to immunize while maintaining a robust cellular immune response. We have shown that immune responses in mice immunized with low dose adenovirus can be boosted with a 10 fold higher dose of virus, and that the induced immune responses are enhanced by the presence of MPL in the primary immunization.

These studies have also been extended to include priming with DNA. Mice primed with a single dose of TbH9 plasmid DNA and boosted with adenovirus show a moderate increase in CD8+ T cell responses when compared to administration of virus alone, as measured by ELISPOT. In contrast, boosting with adenovirus plus MPL after DNA priming shows an enhancement in both CD8+ and CD4+ T cell responses.

In summary, the use of a TLR-4 agonist, such as MPL, can adjuvant T cell responses induced by vaccination with adenovirus over a range of doses and routes of administration tested. Antibodies that bind adenovirus are increased in titer by the addition of adjuvant to the formulation, but this is not accompanied by a significant increase in neutralizing antibodies. The use of MPL allows for immunizations with lower doses of adenovirus while maintaining robust antigen-specific responses. This formulation has several potential benefits: (1) a lower primary dose allows for increased efficacy of a secondary adenovirus immunization, due to low or undetectable levels of adenovirus neutralizing antibodies; (2) increased safety due to inoculation of lower numbers of adenovirus particles; (3) potentiation of DNA prime-adenovirus boost strategies.

A20

Determination of the optimal form of a DNA vaccine for antigen presentation to induce an effective humoral and cell mediated response against a B-cell lymphoma.

Idiotypic determinants can act as tumor-associated antigens for B-cell lymphoma. Naked plasmid DNA vaccines encoding these determinants provide a promising alternative to protein immunization. With this approach we have amplified the variable heavy and variable light sequences from our tumor model Ig (2C3E1,a B cell hybridoma) fused them together (VH-VL) and cloned the sequence into an expression vector. We have subsequently generated several varieties of this basic vaccine construct, including one that expresses a membrane bound 2C3Ig epitope, one that expresses a secreted version and one wherein the epitope is retained within the cytosol. A set of the above vaccine constructs with a 15 amino acid linker inserted between VH and VL has also been generated. We compared the antibody response to the above constructs along with mouse survival to tumor. A moderate antibody response was detected to all, with the highest response observed with the secretory form with a linker. These results corroborate earlier studies wherein vaccination with idiotypic protein mainly produced anti-idiotypic antibody response. These constructs exhibited little ability to prolong mouse survival to tumor. This work indicates that secretory form with the spacer appears to be better suited for antigen presentation in the context of class II MHC. To determine the same in the context of class I MHC we have compared the CTL responses of the above constructs in terms of their ability to be killed by the effector splenocytes from tumor bearing mice, to that of the original parent tumor. Our results indicate that the site of expression of the idiotypic protein (secreted or membrane bound) or the presence of a linker in the above vaccine constructs does not effect antigen presentation for CTL recognition. We are currently trying to further delineate the response to the above vaccine variants in terms of their Th1 or Th2 response by analyzing their cytokine profile *in vitro*. This study can help us develop a vaccine regimen for B-cell lymphoma and also provide insights for other DNA vaccine models.

Differences in Localization of Free and Liposome-Encapsulated Antigens in Macrophages and Dendritic Cells

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The uptake and processing of liposomal antigen follows the classical MHC class I pathway in murine bone marrow-derived macrophages (Mac), whereas free antigens follow the MHC class II pathway. It is unclear if human monocytes (Mono) or dendritic cells (DC) process free and liposomal antigen in the same manner as murine Mac. We have recently demonstrated that depletion of cellular cholesterol interferes with intracellular trafficking of liposome-encapsulated antigens. Since cholesterol and glyco-sphingolipids are major components of lipid rafts, the distribution of lipid rafts was examined by staining with FITC-Cholera toxin. Human peripheral blood-derived DC showed the highest amount of patchiness followed by Mac and Mono. These cells were incubated with free Texas Red-labeled ovalbumin (OVA), a large synthetic Ebola peptide or these labeled antigens encapsulated in liposomes, followed by staining of the trans-Golgi area with NBD-ceramide. While liposomal OVA was transported to the trans-Golgi in greater than 41% of the Mac, free ovalbumin remained spread diffusely throughout the cytoplasm with only 6% localization. Likewise, fewer than 10% of the Mono exhibited trans-Golgi localization with the free antigens. In contrast, the majority of DC localized free antigens in the trans-Golgi (Ebola, 68%; OVA, 83%). Liposomal antigens as expected were localized (55%) in the trans-Golgi area in DC. Thus, these studies demonstrate a major difference in antigen processing/trafficking between human DC, Mono and Macs. The differences observed could be dependent upon the type of antigen processing cell or due to the different mechanisms of uptake of antigens by DC and Macs.

A22

T_H2 and T_C2 effector T cells are not the sole determinants of Respiratory Syncytial Virus-induced eosinophilia

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Respiratory Syncytial Virus (RSV) is an important pediatric pathogen and a major cause of lower airway disease in infants and young children. Despite the magnitude of the clinical problem, no safe and effective vaccine is yet available. This is due in part to the difficulties inherent in immunizing young infants, but work has also been inhibited by the legacy of vaccine-enhanced disease seen following trials of a formalin-inactivated, alum precipitated RSV vaccine preparation that took place in the 1960s. Enhanced disease was eosinophilic in character and associated with a T_H2 cytokine response to viral antigen. We have observed that mice lacking the interferon (IFN) signal transducer STAT1 suffer from enhanced eosinophilic RSV disease following primary infection in the absence of priming.

In infected 129 SvEv STAT1-/- mice, severe eosinophilic lung pathology is associated with IL-5 and IL-13 production from RSV-F specific $CD4^+$ and $CD8^+$ T cells. In light of the important role that IFN-γ and its downstream mediator STAT1 are known to play in development of the T_H1 response, this is not unexpected. To determine whether these effects were entirely due to the lack of IFN-γ effects, we infected a series of 129SvEv mouse strains lacking the IFN-α receptor (IFN-α/β R-/-), the IFN-γ receptor (IFN-γ R-/-), both IFN receptor chains (IFN-α/β/γR-/-), or STAT1. We found pronounced eosinophilic lung disease in mice lacking both IFN receptors or STAT1, but only small numbers of eosinophils in the lungs and BAL fluids of infected IFN-γ R-/- animals. These significant differences in pathology occurred despite the fact that all three of these strains developed T_H2 or T_C2 cytokine responses to RSV-F protein epitopes that elicited T_H1 or T_C1 cytokines from immune wild type or IFN-α/β R-/- splenocytes. We conclude from these studies that although CD4+ T_H2 cell differentiation will occur in the absence of IFN-γ, the presence of an intact IFN-α/β signaling pathway inhibits the development of eosinophilic disease. Supported by NIH grant AI47226 to JED.

A23

Modulation and potentiation of the immune response to DNA vaccines by linear MIDGE vectors coupled to peptides

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The use of linear, minimalistic (MIDGE) vectors containing only the expression cassette, are a safer and more efficient alternative to plasmids at inducing protective immune responses in DNA vaccination protocols. In addition, the covalent attachment to these vectors of a short peptide representing a nuclear localisation signal (NLS) leads to a drastic enhancement of antigenicity, earlier onset of measurable antibody titers and a pronounced shift of the cytokine profile accompanying the response towards the Th1 type. These advantages are being exploited in several applications where a quicker immune response or a Th1-type response is required. Although the mechanism underlying these effects is still unknown, preliminary evidence suggests a direct activation of Dendritic cells by this linear DNA-NLS peptide structure as the most likely candidate. The influence of different peptide sequences on this potentiating effect on DNA vaccines, as well as the structural requirements needed, is currently under investigation and will be further discussed.

Analysis of the Anti-gp41 Specific Immune Response in HIV-1-Positive Sera Using a Multiplexed Antibody Detection Assay

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Abstract : A fluorescent-based, multiplexed, epitope mapping assay was developed to characterize immunogenic and non-immunogenic regions of the HIV-1 envelope glycoprotein gp41. The assay was used to analyze the magnitude, breadth and specificity of the antibody response to recombinant gp41, the fusion peptide region, the N-heptad region, the C-heptad region and overlapping epitopes in the 2F5 and 4E10 regions. In addition, the antibody responses to HIV-1 $gp120_{IIIB}$, gp41-5-helix and 6-helix, gag p24, BSA and internal control peptides were also characterized. Analysis of HIV-positive and negative sera against the panel of HIV-1 gp41 peptides and HIV proteins revealed a broad range of patient specific immune responses and revealed that there are no immunologically silent regions in HIV-1 gp41. These same sera were also tested in single-cycle and multi-cycle infectivity assays to determine whether there was a correlation between the antibody-binding profile and the HIV-1 neutralization titer. Using this methodology there was no apparent correlation between either the breadth or the magnitude of the patient sera antibody response to HIV-1 gp41 and the neutralization potency of the sera. This multiplex Luminex based technology allows for the rapid analysis of complex immune responses to viruses and bacteria for epidemiologic, therapeutic antibody and vaccine efficacy studies.

A25

Indoleamine 2,3-Dioxygenase Expression is Increased in Immature Human Dendritic Cells Exposed to Melanocyte Derived gp100

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Dendritic cells (DC) can initiate both immunostimulatory and tolerogenic responses. Indoleamine 2, 3-dioxygenase (IDO) is a tryptophan-catabolizing enzyme believed to suppress T-cell proliferation in pathophysiological conditions and promote tolerance. One regulatory point in the development of immune responses involves the interaction of T-cell with DC's. Monocyte derived immature DC's stimulated with LPS increase their expression of MHC class II, CD86, but also IDO, while displaying an activated immunogenic phenotype. We have begun to explore this apparently contradictory capacity of DC to express both T-cell activating and inhibitory activities. Our recent studies have shown that most autoantigens, including the melanocyte antigen gp100, are chemoattractants for monocyte derived immature DCs. However, these autoantigens do not activate DC's. Culturing iDC with gp100 does not result in activation of DC's as measured by increased surface expression of HLA-DR, CD86 or CD40. However, gp100 treatment does increase IDO mRNA expression in a dose dependent manner. IDO induction may provide a mechanism, by metabolizing tryptophan, by which antigens normally suppress immune responses. Consequently, overcoming the IDO effect with combinations of autoantigen and cytokines that activate DC may break the tolerogenic state and lead to autoimmunity.

Coxsackievirus B3: Analysis of a Recombinant RNA Vaccine for its Protective Effect against Subsequent Virus Challenge in Mice

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Coxsackieviruses, members of the picornavirus family and the enterovirus genus, are important human pathogens which cause a number of clinically relevant diseases including myocarditis and cardiomyopathy, pancreatitis, meningo-encephalitis, and a variety of less severe diseases. Coxsackievirus B3 (CVB3), which is a non-enveloped plus-stranded RNA virus, is the leading cause of viral myocarditis and may be involved in around 50% of acute myocarditis cases and 25% of dilated cardiomyopathy cases. Despite the very high morbidity and mortality associated with CVB3 infections, so far, there is no vaccine against the virus.

We altered the infectious CVB3 clone pH3 by site-specific mutagenesis based on PCR, to produce RNA carrying mutations in the cleavage site between proteins 2A and 2B within the viral polyprotein. Transfection of this mutated pH3IH1 RNA into HeLa cells, or injection of RNA into mice, should produce CVB proteins and empty capsids, but no functional virus. Therefore, all arms of the immune system should be stimulated, while avoiding the risk of disseminated infection; these qualities should be ideal for a candidate vaccine.

In vitro analysis revealed that transfection of HeLa cells with the pH3IH1 RNA did not lead to the generation of functional virus, whereas transfection of wild-type (wt) RNA yielded abundant infectious viral particles. To test the safety of the RNA vaccine *in vivo*, mice were injected i.m. with 15 µg of pH3IH1 RNA or wt RNA, and viral titers as well as pancreatic histology were analyzed 5 to 9 days post-injection. In mice injected with wt RNA, virus titers were usually very high, causing severe damage of the acinar cells or rarely intermediate, correlating with limited pathological signs. In contrast, no pathological damage was found in any mouse inoculated with pH3IH1 RNA. However, very low viral titers were found in ~25% of these individuals, and sequencing of that isolated virus revealed a full reversion to wt virus. In a preliminary *in vivo* experiment, mice were either left untreated or injected with 1×10^6 pfu of a non-lethal recombinant CVB3 three weeks prior to infection with 8,000 pfu of wt CVB3. Analysis of viral titers in feces, survival over a period of 4 weeks, and the histology of pancreas afterwards indicated that there was a substantial protection of mice injected with the recombinant CVB3 before challenge.

We have initiated studies of the in vivo immunogenicity and protective efficacy of a vaccine comprising pH3IH1 RNA.

The retinal autoantigens, Interphotoreceptor retinoid-binding protein (IRBP) and S Antigen, are chemotactic for human T lymphocytes and immature Dendritic cells

Huifang Dong, De Yang, Saobo Su, Rachel R. Caspi, O.M. Zack Howard, and Joost J. Oppenheim

Abstract:

Bovine IRBP and S Ag are both immunologically privileged retinal antigens that can elicit experimental autoimmune uveitis (EAU) in mice. S Ag is also an autoantigen in Behcet's disease. We show here that IRBP and S Ag are chemotactic for human T lymphocytes, immature dendritic cells (iDC), but not monocytes or neutrophils. The chemotactic actions of IRBP and S Ag are inhibitable by pertussis toxin and they therefore use G protein coupled receptors. Polyclonal antibodies to IRBP and S Ag inhibited the chemotactic activity of IRBP and S Ag respectively. Thus, the autoantigens IRBP and S Ag, which participate in the development of EAU, may recruit lymphocytes and dendritic cells and by interacting with receptors on iDC and T cells may also induce and promote adaptive immune responses.

II

Bioterrorism Vaccines

B1 **TCR contact/minor anchor residues act cooperatively to control peptide binding to HLA-DR1 but dominantly influence stability in the presence of HLA-DM**

Anderson, M. and Gorski, J.
Blood Research Institute of the Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin 53233 and Department of Microbiology and Molecular Genetics, Medical College of Wisconsin, Milwaukee, Wisconsin 53226.

B2 **Synthetic Peptide Vaccine Strategies for the Induction of Robust Antibody and Cytotoxic T Cell Responses**

Naveen K. Dakappagari, Pyles, J, Sundaram, R, Parihar, R, Carson, W. E, Young, D.C, and Kaumaya, P. T. P from the Departments of Obstetrics and Gynecology, Microbiology, Molecular Virology, Immunology, Genetics, Surgery and Biostatistics, Arthur G. James Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210 USA.

B3 **Persistent infection with Ebola virus under conditions of partial immunity**

Manisha Gupta[1], Siddhartha Mahanty[2], Patricia Greer[3], Shieh Wun-Ju[3], Sherif R Zaki[3], Rafi Ahmed[4] and Pierre E Rollin[1]
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B4 **Immunological Bioinformatics**

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www.cbs.dtu.dk.

B5 **MHC class-I epitope prediction from very small datasets**

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B6 **VP22 Enhances Antibody Responses from DNA Vaccines but not by Intercellular Spread**

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B7 **A Surrogate T-Cell Marker for Biodefense Vaccine Development: The Cylex *in vitro* CMI Technology**

Woodcock JB, Kowalski RJ and Britz JA

B8 **Anthrax Vaccination: A Source for a Panel of Potent Fully Human Monoclonal Antibodies using Xenerex Technology.**

Phillip R. Morrow, Ivy Jiang, Shu Man Sun, Rebecca Nedellec, Ritsuko Sawada, Paul Ruther, Fei Wang, Diane Millis, Alejandro Alvarez, John Abrams, & Angray S. Kang
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B9 **Development of Vaccines Against Bacterial (*Bacillus anthracis*) and Viral (HIV) Infections Using A Novel Bacteriophage T4 Multicomponent Display System**

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B10 **PREVENTION AGAINST BIOTERRORISM: PREFACE**

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B1

TCR contact/minor anchor residues act cooperatively to control peptide binding to HLA-DR1 but dominantly influence stability in the presence of HLA-DM

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Abstract

Molecules of the major histocompatibility complex (MHC) are characterized by their polymorphism and by the ability of most MHC alleles to bind a diverse repertoire of different peptides. A better understanding of the mechanism by which such binding complexity can occur should lead to better approaches to vaccine design. Two forces predominate in the binding process; hydrophobic interactions between certain side chains of the peptides and pockets within the MHC, and a network of hydrogen bonds between the peptide backbone and the MHC. Peptide binding is often framed in the context of the dominance of "major" peptide anchor interactions at the 1,4,6 and 9 pockets in the MHCII protein. Here we examine peptide-MHC interaction in the context of a cooperative binding model, by focusing on the effect of peptide residues 2,3,7, and 10 that exhibit a greater degree of solvent accessibility, and are thus also candidates for interaction with T cell receptor (TCR). Using the binding of an influenza derived peptide HA (306-318) to the class II MHC molecule HLA-DR1, we show that changes in the "minor" peptide anchors can have a profound influence on both the binding of the peptide to DR1 and on the editing of the complex by HLA-DM. Furthermore, changes in binding energy as a result of multiple substitutions at these positions indicate an exponential relationship rather than a multiplicative one, in keeping with a cooperative binding model. Our data suggest a more complex mode of peptide binding in which even minor differences in the structure of the peptide epitope can have profound changes. The ability of putative TCR contact residues to display such a restriction on the peptide-MHC interaction could indicate that peptide epitope generation may constrained in a manner that optimizes possible TCR interactions.

Synthetic Peptide Vaccine Strategies for the Induction of Robust Antibody and Cytotoxic T Cell Responses

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Synthetic peptides vaccines have been shown to have a very good safety profile and when used with appropriate adjuvant can elicit long lasting immunity in humans. Recent advances in the synthetic methodologies all for manufacturing them on very large scale at relatively low cost. Therefore, we have focused our efforts designing peptides that are capable of eliciting strong antibody and cytotoxic T cell (CTL) responses in both out bred and in bred strains of rabbits and mice. In the present study, we selected the tumor antigen, HER-2/neu for evaluating our peptide vaccine approaches.

HER-2 is a member of epidermal growth factor receptor family expressed at high-levels in 20-30% breast, ovarian, colon and multiple other human cancers. Using computer based algorithms; we have identified twelve B-cell epitopes and twenty CTL epitopes in the HER-2 protein. To induce high levels of antibodies in an out bred population, each HER-2 B-cell epitope was synthesized collinearly with a universal T-helper cell epitope from measles virus fusion protein. These chimeric B-cell epitope based peptide vaccines elicited high levels of antibodies (titers >100,000) in both out bred rabbits and mice. Antibodies induced by two chimeric B-cell epitope vaccines (316-339 and 628-647) selectively inhibited the growth of HER-2 positive tumor cells *in vitro* and in solid and metastatic mouse models of HER-2 tumorigenesis. Furthermore, a combination of multiple B-cell epitope vaccines and interleukin-12 showed enhanced immunogenicity and caused the greatest reduction in the number of pulmonary metastases. Likewise, to induce polyclonal CTL responses, a peptide construct incorporating multiple CTL epitopes in tandem with arginine spacers was designed to facilitate optimally processing of the individual epitopes and break the formation of junctional epitopes. This novel vaccine construct induced polyclonal CTL responses in HLA-A2.1 (HHD) transgenic mice.

The peptide strategies discussed herein may be applicable for the development of safe and effective vaccines against the emerging infectious disease agents.

B3

Persistent infection with Ebola virus under conditions of partial immunity

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ABSTRACT

Outbreaks of Ebola virus hemorrhagic fever occur periodically in humans and are associated with a high mortality (up to 90%). In general, the outcome of infection is either an overwhelming infection leading to the death of the host, or alternatively, clearance of the virus and complete recovery of the host. The mechanisms by which the virus is cleared in survivors and the correlates of protective immunity to Ebola are not well defined. In this study, using a mouse model, we determined the role of different arms of the immune system in clearance of Ebola virus and protection against fatal disease. Our data shows that 100% of mice deficient in β2-microglobulin (CD8 T cells) succumb to subcutaneous (sc) infection with high tissue viral titers, whereas mice deficient in B cells or CD4 T cells survive and clear the infection. This suggests that CD8 T cells play a critical role in protection against subcutaneous infection and that neither CD4 T cells nor antibodies are required for this protection. B cell-deficient mice that survive the primary sc infection (referred to as "vaccinated" mice) transiently depleted or not depleted of CD4 T cells also survived lethal intraperitoneal (ip) re-challenge for up to 25 days and controlled the infection similar to "vaccinated" +/+ mice. In contrast, 100% of "vaccinated" B cell-deficient mice that were depleted of CD8 T cells had high viral titers in all tissues tested following ip re-challenge and succumbed to infection within 6 days, suggesting that memory CD8 T cells by themselves can protect mice from early death. Surprisingly, "vaccinated" B cell-deficient mice, after initially clearing the infection, were found to have viral antigens in tissues later (day 120-150 post ip infection). Furthermore, following ip re-challenge, "vaccinated" B cell-deficient mice that were transiently depleted of CD4 T cells had higher levels of viral antigen and earlier (days 50-70) than "vaccinated" undepleted mice. This demonstrates that under certain conditions of immunodeficiency Ebola virus can persist and that the loss of primed CD4 T cells accelerates the course of persistent infections. Taken together, these data show that CD8 T cells play an important role in protection against acute disease while both CD4 T cells and antibodies are required for long-term protection. These observations provide the first evidence of persistent infections by Ebola virus and suggest that under certain conditions of immunodeficiency a host can harbor Ebola virus for prolonged periods, potentially acting as a reservoir for the maintenance of Ebola virus in nature.

Immunological Bioinformatics

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New experimental methods such as sequencing, DNA array, and proteomics generate huge amounts of data and there is an ongoing need to develop new methods for handling these large data sets. Immunological Bioinformatics is the field of applying and developing bioinformatics tools in the area of immunology. The long term goal of the research is to establish an in silico immune system. This may be done in a stepwise fashion where models are developed for components of the immune system. These models can be combined and may help to understand diseases, and develop therapies, vaccines and diagnostic tools for diseases such as HIV, TB, allergy and cancer.

The Immunological Bioinformatics group at CBS is developing computational methods that can aid in the search for new vaccines and therapies. The immune system does not react to entire pathogens but rather to parts of these called epitopes. A major aim of the group is to develop methods that can be used to identify epitopes in genomic data and to select which epitopes should be present in a vaccine.

Current projects include:

- development of accurate methods for predicting peptide binding to HLA-A2 as well as to other Class I and Class II HLA molecules,
- prediction of B cell epitopes,
- optimization of plasmids containing multiple epitopes,
- proteasomal cleavage site predictions, and
- epitope/pathogen database construction
- An online server, NetChop, has been developed for prediction of proteasomal cleavage sites, and a neural net based method for predicting peptide binding to HLA-A2

In the Fall 2002, the group held a first course (masters/PhD level) in Immunological Bioinformatics (http://www.cbs.dtu.dk/courses/27485.imm/)

B5

MHC class-I epitope prediction from very small datasets

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Abstract

The public available peptide – MHC affinity prediction methods are all limited to the MHC alleles that are the most common in selected populations. Even though several alleles can be pooled into supertypes, which have overlapping peptide specificities, these supertypes are not always well defined, and the peptide profile for different alleles within a single supertype will often differ significantly.
A method for predicting the affinity of peptides to specific MHC class I alleles have been developed. Position specific scoring matrices is calculated using advanced multiple alignment protocols. The output from these matrices and the sequence information, combined with the usage of randomly selected assumed negatives is subsequently used to train artificial neural networks. Using this procedure with MHC binding information from less than 30 peptides leads to a predictive performance comparable to public available predicting schemes, highly expanding the allele space for peptide prediction. Further more can the output from the neural networks easily be converted to a quantitatively estimate of the affinity.

VP22 Enhances Antibody Responses from DNA Vaccines but not by Intercellular Spread

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DNA vaccines have been successful inducers of protective immune responses against many pathogens in small animal models. So far however, these immune responses have proved insufficient in primates. Strategies which increase protein expression, the distribution of the antigen or more specific targeting of the antigen could lead to increased immune responses. The VP22 protein, a tegument component of the Herpes Simplex virus type I, has been described as exhibiting the property of intercellular spread. This translocation protein is exported from the cells in which it is synthesised, despite lacking a signal sequence, by non-classical Golgi-independent secretion. Following export, the VP22 protein may enter the surrounding cells with high efficiency. Fusion proteins of VP22 plus an antigen may also facilitate cell to cell spread.

Our hypothesis was that the inclusion of the VP22 protein within the vector of a DNA vaccine may lead to the enhanced distribution of the expressed protein, possibly resulting in increased immune responses. The gene for the green fluorescent protein (GFP) was encoded in a DNA vaccine either alone or as a fusion with the VP22 gene. Following transfection into COS-7 cells in which the cells were fixed with methanol for analysis, an increase in the numbers of cells expressing the fusion protein VP22-GFP was noted with almost 100% of cells exhibiting the expressed protein.

However, the exact mechanism by which VP22 mediates intercellular spread remains to be elucidated, and recently there have been suggestions that VP22 does not mediate intercellular spread at all. Therefore studies were conducted in live COS-7 cells to closely examine the transfection patterns of the expressed proteins. The distribution of the GFP protein following expression of a DNA vaccine in COS-7 cells was predominantly cytoplasmic as assessed by confocal microscopy. In contrast, in cells transfected with DNA vaccines expressing VP22-GFP, the protein was located in the nuclei of cells. However, no differences were noted in the relative numbers of cells expressing the respective proteins by either confocal microscopy or FACS analysis. Furthermore, a known number of cells expressing each protein were isolated by a FACS sorter and co-plated with untransfected cells. Following incubation and recounting, there was no evidence that the VP22-GFP protein had spread into untransfected cells indicating no intercellular spread.

Following vaccination of mice with the DNA vaccines, increased antibody responses specific to GFP were noted in the group immunised with DNA expressing VP22-GFP compared to GFP alone. These results suggest that although VP22 may enhance *in vivo* immune responses, the mechanism appears not to be intercellular spread and further work needs to be done to fully elucidate how these immune responses are increased.

B7

A Surrogate T-Cell Marker for Biodefense Vaccine Development: The Cylex *in vitro* CMI Technology

Woodcock JB, Kowalski RJ and Britz JA

As the threat of bioterrorism heightens, needs for vaccines against many bio-warfare agents are rapidly coming to the forefront of research and development. Infection with pathogenic organisms results in immune responses to a broad spectrum of epitopes presented to T-cells by antigen-presenting cells. Vaccination is effective prophylactically against many infectious diseases by inducing humoral and cellular host defense mechanisms. Many currently used vaccines elicit antibody responses that provide effective protection; however, antibodies are not effective against all microorganisms (i.e. tuberculosis, malaria, hepatitis C or HIV), and T cell-dependent responses are required to prevent disease. Cylex has developed an *in vitro* assay that directly measures antigen-specific cellular immune responses of CD3+ lymphocytes that has direct application to the assessment of vaccine efficacy. The Cylex *in vitro* CMI assay employs proprietary co-stimulation factors and magnetic selection of CD3+ cells from whole blood to measure immune cell function in less than 24 hours after *in vitro* exposure to antigens. After incubating a whole blood sample with the antigen, CD3+ cells are magnetically selected, washed and lysed to release intracellular ATP, which is quantified using a calibration curve. Increases in the intracellular ATP of the CD3+ cells correlate with T-cell activation and provide a standardized measure of immune cell function. For this study, the immune response of individuals to foreign antigens including Anthrax, Influenza, CMV and Tetanus was determined. Volunteers with known natural exposure or vaccination showed significant responses; whereas, those not exposed or unvaccinated showed no response. Unlike traditional methods, this technology allows rapid assessment of cell-mediated immunity to antigenic challenges *in vitro* and thus the functional activity of their T-cells.

Anthrax Vaccination: A Source for a Panel of Potent Fully Human Monoclonal Antibodies using Xenerex Technology.

Phillip R. Morrow, Ivy Jiang, Shu Man Sun, Rebecca Nedellec, Ritsuko Sawada, Paul Ruther, Fei Wang, Diane Millis, Alejandro Alvarez, John Abrams, & Angray S. Kang
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Antibodies that bind to the protective antigen (PA) component of the tripartite anthrax exotoxin provide protection as single agents. Here we describe the generation of a series of fully human anti-anthrax protective antigen (PA) toxin antibodies derived from PBMC obtained from Anthrax Vaccine Adsorbed (AVA) vaccinated donors. The antibodies were obtained through the combined use of in vivo immunization of SCID mice reconstituted with human PBMC, and subsequent recovery and immortalization of engrafted cells (Xenerex Technology). From a series of anti-PA antibodies generated from the engraftment experiments, one fully human monoclonal antibody was selected as a lead candidate (AVP-8C1) based on its in vitro ability to protect RAW 264.7 mouse macrophage cells from toxin challenge. The in vitro neutralizing efficacy (IC_{50}) of AVP-8C1 was determined to be approximately equimolar with the input PA toxin concentration used in the bioassay. The equilibrium dissociation constant (K_d, K_{on} & K_{off}) determined by BiaCore and KinExA were in close agreement. The amino acid sequence for the lead monoclonal antibody AVP-8C1 deduced from heavy and light chain cDNA sequencing permitted germline V region identification. Extensive somatic hypermutations were observed in both chains.
The lead antibody AVP-8C1 was found to confer full protection *in vivo* in a recombinant PA toxin-challenge prophylaxis model in rats. This potent fully human anti-PA toxin protective antibody is a very attractive lead candidate for further studies aimed at evaluating suitability for human use in vivo for prophylaxis and immediate post-exposure treatment against intentional release of aerosolized Bacillus anthracis spores.

Development of Vaccines Against Bacterial (*Bacillus anthracis*) and Viral (HIV) Infections Using A Novel Bacteriophage T4 Multicomponent Display System

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The icosahedral capsid of bacteriophage T4 offers very attractive features for development of multicomponent vaccines. Two nonessential proteins, Hoc (highly antigenic outer capsid protein) and Soc (small outer capsid protein) are displayed at a high density on the T4 capsid surface. Foreign antigens can be fused to the termini of these proteins and displayed on the capsid surface. Previously we have shown that mice immunized with a 36-amino acid PorA peptide from *Neisseria meningitidis* fused to Hoc or Soc and displayed on T4 capsid elicited strong PorA-specific Ab responses in the absence of any adjuvant. In this study, we have used this system for displaying HIV and anthrax antigens.

Recombinant HIV-P24-Hoc, HIV-Tat-Hoc, and Anthrax protective antigen (PA)-Hoc were constructed, overexpressed in *E. coli*, and purified. The purified proteins were loaded onto phage T4 capsid using a novel *in vitro* assembly system. The display of the fusion proteins on phage T4 nanoparticles and their copy number were characterized by SDS-PAGE and Western blotting. All proteins, including the 83 kDa full-length anthrax PA were assembled efficiently on the capsid surface.

The P24-T4 nanoparticles were tested for their immunogenicity. BALB/c mice were immunized with free or liposome-encapsulated T4 particles displaying Hoc-P24. Antibody and cellular responses were determined. HIV P24-specific IgG endpoint titers of >200,000 were obtained with both the free and the liposome-encapsulated antigens. The titers were maintained even 6 weeks after the last boost. Stimulation indices of 6 were obtained with the spleens from the two groups of mice. P24-specific IL-4 and IFNg secreting cells were detected in the spleens and lymph nodes.

P24-Hoc displayed on the T4 capsid elicited strong humoral and cellular immune responses even in the absence of an adjuvant. The induced antigen-specific IgG Abs exhibited high titers and are long-lasting. Recombinant T4 nanoparticles displaying full-length HIV P24/Gag, Tat, and P120/Env, can be developed as an efficacious multicomponent vaccine against HIV. Similarly, the T4 nanoparticles displaying PA, LF (lëthal factor), and EF (edema factor), can be developed as an efficacious multi-component anthrax vaccine. Thus, the phage T4 system is being developed as a novel platform for construction of these future generation vaccines.

PREVENTION AGAINST BIOTERRORISM: PREFACE

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The proposed strategy permeating our plan -- for preventing the effects of sudden biologic and/or chemical, bioterristic attacks-- is based on our discovered and US-patented methods for long-term suppression of antigen-specific immune responses (i.e., suppression of both Abs and CTLs) to well-defined, low and high molecular weight immunogens, as well as to their covalent conjugates, for extended periods (viz., up to a minimum of 540 days, which was the longest period for one of our earliest published experiments, utilizing inbred mice.)

We suggest that - for discerning our method and for testing the appropriateness and feasibility of our above-mentioned plan, for defence against biological and chemical bioterrorism, - it would be advisable to first scan through our accompanying attachments, which represent the foundation and characteristic features of our antigen-specific, immunosuppressive and long-lasting methods.

We anticipate that our procedure could be adapted for passive protection of humans of all ages to

(i) bacterial and viral diseases, before or after their having been infected with the corresponding bacterium or virus, which cannot be inactivated unless it is subjected to dangerous drug(s), which would also afflict deleteriously the infected humans, and

(ii) natural and synthetic poisons.

In this connection, it is recommend that :

Firstly, for protection of individuals of all ages, the population at risk be pre-tolerized to each of the three mentioned anti-virus antibodies, by injection of each of the three corresponding mPEGylated (i.e., tolerogenic) conjugates, which would be synthesized as described in the Poster. Finally, the so-treated individuals could be "flooded" with each of the three corresponding anti-virus antibodies.

Subsequently, protective antibodies to chemically inactivated viruses or bacteria, or to poisons, be produced in large quantities in horses and/or goats[♣]. Thereafter - for the security of maintaining a large and continuously reproducible source of the Fab regions of these abs over indefinite periods - it would be advisable that the Fab regions be copied by established, recombinant technology methods.

[♣] ***Horse abs had been successfully used in humans.***

III

Bacterial Vaccines

C1 **Safety and efficacy of intramuscular and intranasal vaccination of calves against haemorrhagic septicaemia with an attenuated *aroA* derivative of *Pasteurella multocida* B:2**

*J. Christopher Hodgson[1], Anna Finucane[2], Mark P. Dagleish[1], Roger Parton[2] and John Coote[2]**
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C2 **Distribution of Adsorbed Antigen in Mono-Valent and Combination Vaccines**

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C3 **Th1 cell antigens and peptides encoded by the RD1 genomic segment of *Mycobacterium tuberculosis***

Mustafa AS, Hanif SNM, Shaban F and Al-Attiyah R
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C4 **Isolation of porin-like proteins from *Burkholderia mallei* for possible vaccine candidates.**

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C5 **AUTOMATED SYNTHESIS OF OLIGOSACCHARIDE ANTIGENS TO ENABLE VACCINE DEVELOPMENT: AN ANTI-TOXIN MALARIA VACCINE CANDIDATE AND OTHER EXAMPLES**

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C6 **Localization of BmpA in *Borrelia burgdorferi* during growth *in vitro* and *in vivo***

Junghee J. Shin, Henry P. Godfrey, and Felipe C. Cabello
Microbiology / Immunology Department, New York Medical College, Valhalla, NY

C7 **Glycosylphosphatidylinositol Structures as Anti-toxin Malaria Vaccine Candidates via Solution and Solid-phase Synthesis**

Daniel A. Snyder, Regina L. Soucy, Yong-Uk Kwon,
Michael C. Hewitt, Peter H. Seeberger
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C8 **Evaluation of Recombinant *Listeria monocytogenes* as a Vaccine Vector**

Rosemary Stevens, Alora LaVoy, Gregg Dean
North Carolina State University, Raleigh, NC, USA

C9 **COMPUTATIONAL TOOLS FOR IDENTIFICATION OF POTENTIAL T CELL EPITOPES**

Myong-Hee Sung and Richard Simon
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National Institutes of Health

Safety and efficacy of intramuscular and intranasal vaccination of calves against haemorrhagic septicaemia with an attenuated *aroA* derivative of *Pasteurella multocida* B:2

J. Christopher Hodgson[1], Anna Finucane[2], Mark P. Dagleish[1], Roger Parton[2] and John Coote[2]*

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Haemorrhagic septicaemia in South Asia is a fatal systemic disease of cattle and buffalo caused by infection with *Pasteurella multocida* serotype B:2. Existing bacterin vaccines are oil-based, difficult to use and of limited duration and the disease remains a significant obstacle to sustainable agriculture in the region. Vaccination with a live attenuated *aroA* derivative of *P. multocida* B:2 has been shown to be highly protective in mice but the safety and efficacy of use in the target species has not been tested. In a pilot study two groups of 4 calves were immunised at 2 and 6 weeks old with approximately 10^9 cfu of an *aroA* derivative either intranasally (Group 1) or intramuscularly (Group 2). Both groups of calves and 3 control calves (no vaccination) were challenged subcutaneously at 8 weeks old with 10^7 cfu of the virulent parent strain. Calves were monitored at regular intervals each day during the experiment for any effect of treatment, and dullness and increased temperature were the main indicators used to detect the onset of disease. Blood samples (10 ml) from a jugular vein were collected from calves before and at intervals after challenge and serum analysed for the acute phase proteins haptoglobin and serum amyloid A.

Vaccination caused no significant change in rectal temperatures of Group 1 calves but Group 2 calves developed a significant ($P<0.05$) febrile response within 3h of each vaccination that returned to normal following treatment with anti–inflammatory drugs. All four calves in Group 2 were solidly immune to virulent challenge, showing no significant clinical, biochemical or pathological responses. Rectal temperatures of control and Group 1 calves increased significantly ($P<0.05$) within 6h of challenge and the calves became dull, with some showing increased respiratory effort and slight nasal discharge. Additionally, serum amyloid A concentrations increased between 10 and 24h following challenge. These calves were treated with anti–inflammatory drugs but the clinical condition of 2 control and 3 Group 1 calves did not improve and they were killed for humane reasons. Tissues from these and calves killed on schedule at the end of the experiment were taken for histopathological examination. Pathological evidence consistent with haemorrhagic septicaemia was found in control and Group 1 calves but not in Group 2 calves.

The work indicated that intramuscular vaccination of calves with a live attenuated *aroA* derivative of *P. multocida* B:2 was 100% effective in preventing disease following challenge with the virulent parent strain but that treatment with anti–inflammatory drugs was necessary to offset the febrile response to vaccination via this route. Further work will investigate the safety and efficacy of intramuscular vaccination using lower doses of vaccine.

C2

Distribution of Adsorbed Antigen in Mono-Valent and Combination Vaccines

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[a]Industrial & Physical Pharmacy, [b]Veterinary Pathobiology, and [c]Basic Medical Sciences
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Content uniformity of mono-valent and combination vaccines in which the antigen is adsorbed by aluminum hydroxide adjuvant (AH) was studied. AH has a complex morphology and is present in vaccines as 1-10 μm aggregates composed of fibrous primary particles (4.5 nm x 2.2 nm x 10 nm). Bovine serum albumin (BSA) and alpha-casein (AC) were the model antigens. For the mono-valent vaccine systems each model antigen was labeled with a green fluorescent dye to follow adsorption to AH. BSA adsorbed through electrostatic attractive forces while AC, which contains 8 phosphate groups, adsorbed through ligand exchange of phosphate with hydroxyl at the adjuvant surface. When combined with AH both model antigens adsorbed to the adjuvant within one minute. Over time uniform surface coverage of the adjuvant was observed in both systems.

For the combination vaccine systems, one sample of AC or BSA was labeled with a green fluorescent dye and another sample with a red fluorescent dye. Both antigens were adsorbed separately to AH and then combined. After combination, new aggregates were observed within 30 min. that exhibited well defined red and green regions. This indicated that the original aggregates with adsorbed AC or BSA were dissociating and then recombining to uniformly distribute each antigen throughout the vaccine. After 60 min. the red and green regions of the aggregates became smaller and more numerous as the dissociation and re-aggregation process continued. In conclusion, as long as adequate mixing procedures are followed content uniformity should not be an issue when adsorbing an antigen to aluminum hydroxide adjuvant or when combining separate vaccines into a combination vaccine.

C3

Th1 cell antigens and peptides encoded by the RD1 genomic segment of *Mycobacterium tuberculosis*

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RD1, a 9.5 kb genomic DNA segment of *Mycobacterium tuberculosis* is deleted in all the vaccine strains of *Mycobacterium bovis* BCG. To identify antigens encoded by the RD1 region as candidates for a new vaccine against tuberculosis and reagents for specific diagnosis, the present study was carried out to identify the major Th1 cell antigens and immunodominant peptides of *M. tuberculosis* encoded by genes predicted within the RD1 segment. Peripheral blood mononuclear cells (PBMC) were obtained from the blood of tuberculosis patients (n = 30) and healthy subjects (n = 40) by density gradient centrifugation on Lymphoprep. The isolated PBMC were tested in antigen induced proliferation and IFN-γ release assays with complex (whole cell *M. tuberculosis* and culture filtrate) and single cross-reactive (MPB70) mycobacterial antigens and peptides corresponding to the proteins encoded by the genes predicted in the RD1 segment. A total of 220 peptides were synthesized by using fmoc chemistry and tested as a master mix of all the peptides (RD1mix). Peptide pools corresponding to individual proteins and single peptides of the major and moderate antigens were also tested in both the assays for Th1 cell reactivity. Genomic HLA-DR and -DQ typing of PBMC was performed to confirm the HLA heterogeneity of the donor groups and promiscuous presentation of the immunodominant peptides. The results of antigen induced proliferation and IFN-γ secretion from PBMC showed that both tuberculosis patients as well as healthy donors responded strongly to the RD1mix. However, when tested with the peptide mixtures of individual ORFs, tuberculosis patients showed strong responses to ORF3, ORF6 and ORF7, whereas ORF5 and ORF9 gave equivalent responses in tuberculosis patients and healthy donors. The results further showed that the RD1 segment of *M. tuberculosis* encodes three major (ORF5, ORF6 and ORF7) and two moderate (ORF3, ORF9) Th1 cell antigens. When the T cell epitopes of these antigens were mapped by using single peptides covering the sequence of each of these proteins, peptides P3.2 of ORF3, P5.9 of ORF5, P6.4 of ORF6 and P7.1 of ORF7 were found to represent the immunodominant epitopes recognized by Th1 cells from tuberculosis patients, whereas the peptides P5.9 of ORF5, P6.2 of ORF6 and P7.5 of ORF7 represented immunodominant Th1 cell epitopes in healthy subjects. The results of HLA-DR and -DQ typing of PBMC from both the donor groups showed that the tested subjects were genetically heterogeneous. These results suggested that the major antigens of RD1 region and their immunodominant peptides were presented to Th1 cells promiscuously. In conclusion, the study has identified three major and two moderate Th1 cell antigens and the corresponding immunodominant peptides encoded by RD1 DNA segment of *M. tuberculosis*. The antigens and peptides recognized preferentially by tuberculosis patients will be useful in specific diagnosis and those recognized by healthy subjects have potential in developing new vaccines against tuberculosis.

Supported by the Research Administration grant MI 114 and the College of Graduate Studies, Kuwait University, Kuwait.

Isolation of porin-like proteins from *Burkholderia mallei* for possible vaccine candidates.

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Burkholderia mallei, the etiological agent of glanders, is a biological warfare threat agent that causes this potentially fatal disease, primarily by inhalation. *B. mallei* infection can cause lymphadenitis with subsequent involvement of a variety of tissues and organs, possibly including brain and meninges. Limited information is available about the pathogenesis, diagnosis, and treatment due to *Burkholderia mallei* infection. One of the important characteristics of *Burkholderia* species is its resistance to many anti-microbial agents. Studies of cell structure and function may be important in understanding the mechanism and pathogenesis of the disease due to *B. pseudomallei* and related species. There is no effective vaccine available, and the bacteria are resistant to many antibiotics. One reason the cells may be resistant to antibiotics is due to decreased porin-mediated outer-membrane (OMP) permeability. Because of this possibility and their potential use as vaccine candidates, we examined the porins present in *B. mallei*. The *B. mallei* OMP was isolated by fractionation, and was analyzed by SDS-PAGE. Several distinct proteins were observed in the preparation. The purification involved selective extraction of OMP with salts, followed by size exclusion chromatography, using Sephadex G-25, Sephacryl-200 or superose-GF HPLC columns. Purified preparations showed three major peaks of 82, 40, and 32 kDa, similar to a porin protein profile reported for *Pseudomonas cepacia*. Western blot analyses, using a polyclonal anti-rabbit serum or monoclonal antibody directed against *B. mallei,* strongly reacted with the purified OMP, suggesting that cellular protein possessed surface antigenicity. Based on our data, the membranes of glanders, contained a concentrated complex of antigens, we also found that the *Burkholderia* species differed in the antigenic composition of the complex. The results make OMP of *B. mallei* an immunologically significant molecule for study and these immunogenic porin-like proteins may be efficacious as vaccine components.

C5

AUTOMATED SYNTHESIS OF OLIGOSACCHARIDE ANTIGENS TO ENABLE VACCINE DEVELOPMENT: AN ANTI-TOXIN MALARIA VACCINE CANDIDATE AND OTHER EXAMPLES

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Cell surface carbohydrates act as biological markers for various tumors and are involved in parasitic infection. Involvement of carbohydrates in these processes makes them attractive targets for vaccine development. A major impediment to the development of vaccines based on defined carbohydrates has been the lack of pure, structurally defined carbohydrates and glycoconjugates.

Described is the application of an automated solid-phase oligosaccharide synthesizer we developed recently to the preparation of even complex carbohydrates from monosaccharide building blocks.[1] Application of this new synthetic strategy, that should be attractive to the non-specialist, to the assembly of oligosaccharide antigens involved in cancer, infectious diseases, tropical diseases and HIV will be described.

Particular emphasis will be placed on the development of an anti-toxin malaria vaccine using the automated synthesizer to prepare complex oligosaccharides.[2] Work on the development of *Leishmania* and cancer vaccines will also be covered.

1. Plante, O.J.; Palmacci, E.R.; Seeberger, P.H.; *Science* **2001**, *291*, 1523-1527.
2. Schofield, L.; Hewitt, M.C.; Evans, K.; Siomos, M.A.; Seeberger, P.H.; *Nature*, **2002**, *418*, 785-789.

Poster Abstract for Minisymposium III: Bacterial Vaccines

Title: Localization of BmpA in *Borrelia burgdorferi* during growth *in vitro* and *in vivo.*

Authors: Junghee J. Shin, Henry P. Godfrey, and Felipe C. Cabello

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The paralogous *Borrelia burgdorferi bmp* genes (family 36) are located in tandem on the bacterial chromosome. Published evidence of coordinate regulation and differential expression of these genes at the transcriptional level suggested that they might be regulated by environmental stimuli at both transcriptional and translational levels. To test this hypothesis, we raised rabbit antibodies to purified recombinant BmpA (rBmpA) using TiterMax as adjuvant. The titer of antibodies in final bleeds measured against 10 ng of rBmpA in a dot immunobinding assay was $1x10^{10}$. Anti-BmpA immunoglobulin was purified from rabbit sera by ammonium sulfate precipitation and rendered monospecific by repeated absorption with Affi-Gel 10-insolubilized rBmpA. Monospecificity of the absorbed antibody was confirmed by immunoprecipitation with rBmp proteins and by detection of a single spot on immunoblots of two-dimensional nonequilibrium pH gel electrophoresis of *B. burgdorferi* lysates. Immunofluorescence studies with these monospecific antibodies of unfixed *B. burgdorferi* B31 cultured *in vitro* in BSK-H encapsulated in agarose gel microdroplets showed limited surface exposure of BmpA. This limited surface exposure of BmpA was confirmed by its *in situ* partial susceptibility to proteinase K. A similar limited surface exposure of BmpA protein was also detected by partially absorbed anti-rBmpA antibodies in *B. burgdorferi* N40 and 297 strains cultured *in vivo* in BSK-H in dialysis membrane chambers implanted intraperitoneally in rabbits. Anti-rBmpA also exhibited bactericidal activity on *B. burgdorferi* B31 growing *in vitro*. This is consistent with BmpA localization on the exposed outer surface of the spirochete, and suggests inclusion of BmpA in future vaccines for Lyme disease. (Supported by NIH grant R01 AI48856).

Glycosylphosphatidylinositol Structures as Anti-toxin Malaria Vaccine Candidates via Solution and Solid-phase Synthesis

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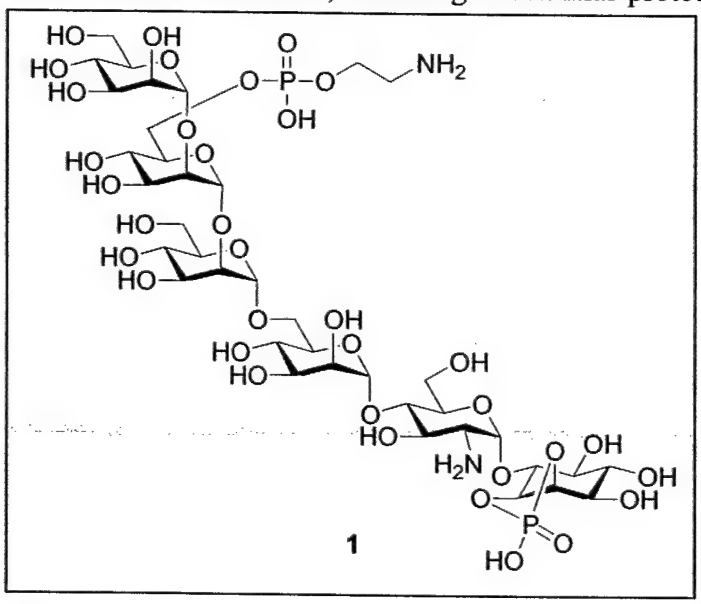
Glycosylphosphatidylinositol (GPI) **1**, the malarial toxin, has shown promise as a vaccine in a rodent model of malaria, affording substantial protection against negative symptomology and fatality,[1] and has been produced by both solution and solid-phase methods.[2] In order to explore the antigenicity of such structures, and to produce a vaccine generating the same range of antibody response as exposure to the natural pathogen, several variants of the GPI structure were synthesized, via new methods; different presentations of the antigen may allow antibody generation targeted to different regions of the GPI, and synthesis of deletion sequences makes possible epitope mapping of antibodies from disease-resistant individuals. Solid-phase techniques should allow for the rapid generation of a wide range of structures.



1

[1] Schofield, L.; Hewitt, M.C.; Evans, K.; Siomos, M.; Seeberger, P.H. *Nature* **2002**, *418*, 785-789.
[2] Hewitt, M.C.; Snyder, D.A.; Seeberger, P.H. *J. Am. Chem. Soc.* **2002**, *124*, 13434-13436.

Evaluation of Recombinant *Listeria monocytogenes* as a Vaccine Vector.

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Listeria monocytogenes (LM) is a gram positive, intracellular organism that has been genetically manipulated to express exogenous proteins and deliver plasmids that encode foreign antigens. LM primarily infects macrophages and dendritic cells and escapes the phagolysosome by secretion of virulence factor listeriolysin-O (LLO). Upon release, LM multiplies in the cytoplasm where it's proteins are presented via the MHC class I pathway leading to a potent immune response. As a strong cell-mediated response is necessary to control many viral infections, *L. monocytogenes* is an ideal vector to present feline immunodeficiency virus (FIV) antigens. Gram positive bacteria have also proven to be an effective carrier for DNA vaccines, thus combining the specific targeting to professional antigen presenting cells with the power of a DNA vaccine.
Full-length FIV *gag* was inserted into the antigen cassette that was flanked by sequences homologous to the LM virulence genes. This plasmid, designated pLMD-gag, was electroporated into wild-type *L. monocytogenes* where stable integration was forced through selective culturing.
Recombinant *L. monocytogenes* (rLM-gag) were then screened for protein secretion by western blot and protein expression in feline macrophages. Next, the envelope from FIV molecular clone $NCSU_1$, was cloned into a eukaryotic expression plasmid, pND14, under control of a CMV promoter. This was transfected into rLM-gag. The resulting rLM-*gag*/pND14-Lc-*env* was shown to express high levels of soluble FIV Gag and Env protein *in vitro*.
Two *in vivo* studies using the FIV Gag-expressing rLM vector have been performed. The first study compares immune response generated by oral versus intravenous vaccination. The second study evaluates cats immunized orally with the Gag-expressing rLM vector transfected with a pND14-Env-LC plasmid. We report the outcome of both studies with an emphasis on T cell responses to FIV Gag and Env peptides as measured by IFN-gamma ELISpot.

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C9

COMPUTATIONAL TOOLS FOR IDENTIFICATION OF POTENTIAL T CELL EPITOPES

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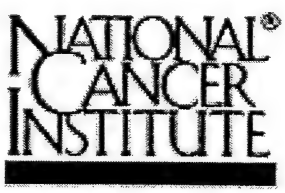
ABSTRACT

We present computational techniques that can be employed to search for potential T cell epitopes from query protein sequences based on information from screening assays using combinatorial peptide libraries and MHC-peptide binding data. Epitope specificity can be considered (i) at the level of the restricting MHC allele or (ii) at the level of T cell receptor (TCR) (see below). Based on a peptide-specificity model of either kind (i or ii), a bioinformatical search can be performed on a query protein database to retrieve high scoring peptides. The search produces a list of potential target peptides that are suitable for experimental evaluation. These computational analyses may accelerate a comprehensive search for the relevant T cell epitopes at initial stages of the vaccine development efforts in infectious diseases and tumor immunotherapy.

For epitope identification problems at level (i), we developed a novel method for constructing statistical models that can predict MHC class I and class II binding peptides. The multivariate models, termed the "peptide property models", use quantitative biophysical parameters of the constituent amino acids. Each MHC-specific peptide property model requires a dataset of sufficiently diverse binding peptides. The model can be used to identify peptides that can potentially stimulate one or more T cell clones whose restricting MHC is that of the model.

A problem at the specificity level of (ii) arises when the goal is to identify the peptides that specifically stimulate the given T cell clone. Positional scanning synthetic combinatorial libraries can be screened in T cell proliferation assays to obtain information on the peptide specificity of the TCR. However, the resulting data requires deconvolution for MHC class II restricted responses due to the complexity of the peptide library mixtures and their MHC binding behaviors. We use mathematical models and simulation to estimate the "true" peptide specificity in the form of a TCR-specific score matrix.



C10

Mucosal DNA vaccination with highly attenuated *Shigella flexneri* protects against a lethal influenza challenge.

William H. Vecino, Natalie Quanquin, William R. Jacobs, Jr and Glenn J. Fennelly

Influenza virus remains a significant cause of morbidity and mortality worldwide in both industrialized and developing nations. Intramuscular immunization does not target the portal of entry for the pathogen. We have previously demonstrated that intranasal attenuated *Shigella* and *Salmonella* harboring DNA vaccines generate humoral (IgG, IgA) and CD8+ responses against measles virus and HIV in mice. Using the same approach, we demonstrated that transiently persistent Δasd *Shigella flexneri* strain 15D harboring an influenza hemaglutinin (HA) DNA vaccine protects against a lethal influenza virus challenge. Mice (n=10) were immunized intranasally X 3 at monthly intervals with 5 X 10^6 c.f.u. of *Shigella* harboring an HA DNA vaccine, intramuscularly with purifed HA DNA vaccine or intranasally with attenuated influenza virus (D2A/WSN/33). Control mice were immunized intranasally with 15D containing the corresponding DNA plasmid without the HA insert, or with PBS. One month after the last immunization mice were challenged by i.n. infection with 10^6 p.f.u of wild type influenza virus (A/WSN/33). Animals were observed and weighed daily for 20 days after challenge. One hundred percent of mice immunized with attenuated *Shigella flexneri* 15D harboring the HA DNA vaccine (9 of 9), attenuated influenza (10 of 10), or intramuscular HA DNA (10 of 10) survived after challenge versus only 30% of control 15D immunized mice ($p<0.05$). This result encourages the further development of *Shigella*, and other attenuated bacteria, as DNA vaccine delivery systems for immunization against influenza and other respiratory viral pathogens.

IV

In Vivo Cell Interaction and Trafficking

D1 **Expression of Human Prostate Specific Antigen (PSA) in Cynomolgus Monkeys Following Intramuscular Administration of a PSA DNA Vaccine**

Patrick Branigan, Nicole Sauers, Deborah Marshall, Joel Cornacoff and Linda Snyder. Centocor Inc., Malvern, PA 19355.

D2 **Protection of tumor growth with adenoviral vaccines expressing HER-2/neu**

Jong M. Park[1], Yoshio Sakai[2], Masaki Terabe[1], Guido Forni[3], John C. Morris[2], Jay A. Berzofsky[1]
Molecular Immunogenetics and Vaccine Research Section[1], and Cancer Gene Therapy Section[2], Metabolism Branch, National Cancer Institute, Bethesda, MD 20892, Department of Clinical and Biological Sciences, University of Turin, I-10043 Orbassano, Italy[3]

D3 **Immune Responses to Human Papillomavirus Antigens in HLA Transgenic Mice**

***Rhonda Kines[1]**, Ellen Lynch[1], Galina Elkin[1], Chella David[2] T.C. Wu[3], Shashikant Lele[4], Kunle Odunsi[4], Yasmin Thanavala[1]*
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D4 **Dissociated expression of IFN-g and of Granzyme-B by HIV specific CD8 cells in infected individuals.**

Thomas O. Kleen, §, Robert Asaad†, Samuel Landry‡, Bernhard O. Boehm§ and Magdalena Tary-Lehmann* *Department of Pathology and †Center for AIDS research,Case Western Reserve University, Cleveland, Ohio 44106, USA; ‡Dept. of Biochemistry, Tulane University School of Medicine, New Orleans Louisiana 70112, USA and the §University Hospital of Ulm, Section of Endocrinology, Ulm, 89081, Germany*

Expression of Human Prostate Specific Antigen (PSA) in Cynomolgus Monkeys Following Intramuscular Administration of a PSA DNA Vaccine

Patrick Branigan, Nicole Sauers, Deborah Marshall, Joel Cornacoff and Linda Snyder. Centocor Inc., Malvern, PA 19355.

Many studies show that DNA vaccines are capable of inducing therapeutic and protective immune responses in a wide variety of preclinical models. In particular, DNA vaccines encoding tumor-associated antigens can elicit protective immune responses in animals, suggesting that this approach may benefit cancer patients as well. We are developing a DNA vaccine (CNTO 23) that encodes human prostate specific antigen (PSA) as a therapeutic for prostate cancer. The purpose of the work reported here was to determine if CNTO 23 plasmid could be detected, and was capable of directing PSA expression, after being injected into the muscles of Cynomolgus monkeys (*Macaca fascicularis*). Nine male Cynomolgus monkeys were divided into three treatment groups receiving 0, 4 or 12 mg injections of CNTO 23. The animals received intramuscular injections of CNTO 23 on days 1, 15, 29 and 43. The doses were divided equally among four dose sites per animal. A sample of muscle from two of the four injection sites was collected via a 4mm-punch biopsy 7 days following the final injection of test article or control article (day 50). Biopsy samples were analyzed by real-time quantitative polymerase chain reaction (PCR) for the presence of plasmid DNA and by reverse transcription-PCR (RT-PCR) and Southern blot analysis for the presence of human PSA mRNA transcripts. CNTO 23 plasmid DNA was consistently detected in 5/6 monkeys injected with CNTO 23, and in none of the control biopsy samples. Additionally, CNTO 23 mRNA transcripts were detected in 4/6 monkeys injected with CNTO 23, and in none of the control biopsy samples. These data demonstrate that CNTO 23 DNA persists at least 7 days following intramuscular injection and is capable of expressing human PSA mRNA transcripts in Cynomolgus monkeys.

D2

Protection of tumor growth with adenoviral vaccines expressing HER-2/neu

Jong M. Park[1], Yoshio Sakai[2], Masaki Terabe[1], Guido Forni[3], John C. Morris[2], Jay A. Berzofsky[1]

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HER-2/neu is an oncogenic tumor-associated antigen overexpressed in a variety of human malignancies such as breast and ovarian cancer. The ability of a HER-2/neu vaccine to protect against tumor growth was assessed in BALB/c mice immunized with recombinant adenoviral vectors expressing HER-2/neu protein (Ad-ECD, extracellular domain only; Ad-TM, ECD and transmenbrane domain). Mice vaccinated with Ad-TM rejected tumor growth when challenged with TUBO cells expressing HER-2/neu, whereas tumor growth ($\geq 250mm^2$) was observed around day 30 after tumor injection into control mice. Ad-TM gave more complete tumor protection than Ad-ECD. While 10^8 pfu of Ad-TM was required to induce the immunity against carcinogenesis in HER-2/neu transgenic mice, which develop spontaneous HER-2/neu expressing mammary tumors, growth of injected TUBO tumor cells was prevented with $10^2 - 10^3$-fold lower doses of vaccine. This may reflect the need to break tolerance in the transgenic model.

In order to see the roles of T lymphocytes in tumor protection, T cells were depleted *in vivo* simultaneously with immunization by Ad-TM. Tumor protection was completely abolished by the depletion of CD4 cells, even though the growth was delayed slightly. However, CD8-depleted mice still could reject TUBO cell challenge, and this rejection was also observed in mice treated with anti-asialo GM1 to see the role of NK cells. The role of CD4 lymphocytes in protective immunity against adoptively transferred tumor cells is very similar to that found in the prevention of carcinogenesis in HER-2/neu transgenic mice. Vaccination of mice deficient of B cells or γ-IFN did not protect from tumor growth. These results show that HER-2/neu- expressing adenoviral vaccines by themselves can protect against tumor growth and protection is very dependent upon CD4 cells, antibodies and γ-IFN.

D3

Immune Responses to Human Papillomavirus Antigens in HLA Transgenic Mice

Rhonda Kines[1], Ellen Lynch[1], Galina Elkin[1], Chella David[2] T.C. Wu[3], Shashikant Lele[4], Kunle Odunsi[4], Yasmin Thanavala[1]

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It is becoming increasingly evident that the need to understand what is occurring at the tumor site is important in designing effective immunologically based therapeutics. Following a viral infection there are multiple factors that influence the immune response elicited to the viral proteins. Some viruses evade the immune system and cause chronic infection. This chronic infection may represent a risk factor for the host to develop cancer (i.e. hepatitis B and C viruses in liver cancer and Human Papillomavirus (HPV) in cervical cancer. A previous study has shown that women who are HLA-DRB1*0401 have an increased susceptibility to develop cervical cancer following infection with high-risk HPV types. The role of HLA and the immune response to HPV could be crucial in clearing the virus before it transforms the host cells. Previous studies have been limited to evaluating immune responses in limited numbers of human subjects of HLA class I transgenic models. HLA class I is down-regulated on many cervical tumors whereas class II has been shown to be up-regulated. We are utilizing an HLA-DRB1*0401 transgenic mouse model to study the HLA-DRB1*0401 restricted immune response to HPV oncogenic protein E7.

Our hypothesis is that HPV infected HLA-DRB1*0401 patients are more susceptible to developing cervical cancer following HPV infection because they are unable to mount an effective immune response to viral antigens. We are studying the immune response to the oncogenic protein E7, as well as overlapping peptides derived from its sequence, in HLA-DRB1*0401 transgenic mice in order to identify potentially immunogenic peptides to be tested in a tumor model. Mice are immunized by either DNA vaccination using the gene gun, vaccinia virus, or whole protein. The DNA and vaccinia virus constructs contain the HPV protein E7 fused to Lysosomal Membrane Protein-1 (LAMP-1). Fusion to LAMP-1 will allow localization of E7 into the lysosomal compartment thus directing it into the class II processing pathway.

Our data indicate a decrease in lymphocyte proliferation to E7 in the DR4 transgenic mice when compared to C57Bl/6 and BALB/c. The DR4 transgenic mice make high levels of IL-10 and IFN-γ in response to secondary stimulation with HPV protein E7 as determined by cytokine ELISA. Further, BALB/c mice develop a stronger and sustained antibody response to E7 compared to the DR4 mice following immunization with E7 DNA via gene gun. Future work will include measuring lymphocyte proliferation and cytokine secretion in response to immunization with DNA and vaccinia virus.

HLA transgenic mice provide us a unique opportunity to understand the immune response to HPV-16 protein E7 and to identify potentially immunogenic peptides for future vaccine studies.

D4

Dissociated expression of IFN-g and of Granzyme-B by HIV specific CD8 cells in infected individuals.

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Unlike naive T cells that do not express IFN-g or Granzyme B, memory CD8 cells rely on these molecules for mediating different effector functions. By secreting IFN-g, CD8 cells locally activate macrophages to engage in host defense. Granzyme B, in contrast, is part of the cytolytic machinery that CD8 cells utilize for perforin-mediated killing of their target cells. In our first set of experiments we show here that immunizations with different adjuvants can induce in mice CD8 cells that differentially express these molecules. Immunization with MHC class I restricted peptides in complete Freund's adjuvant induces IFN-g producing CD8 cells that do not mediate cytotoxicity in chromium release assays. In contrast, injection of the same peptide, in the same dose, but in incomplete Freund's adjuvant, induces CD8 cells that kill, but that do not produce IFN-g (or type 2 cytokines). The former CD8 memory cell type mediates delayed type hypersensitivity, the latter does not induce it. Therefore, in mice, the two discrete CD8 cell memory subpopulations exist whose differentiation relies on different signals, and that serve different effector functions, DTH vs. cognate cytolysis. In our second set of experiment, we tested whether also in humans there is evidence for such differential CD8 cell populations. We studied HIV infected individuals, using HIV peptides to elicit the specific CD8 cells. IFN-g and Granzyme B ELISPOT assays were performed. We found that some peptides induced only IFN-g, in the absence of Granzyme B, while other peptides induced Granzyme B production by CD8 cells, in the absence of IFN-g. The data provide evidence that also in humans these two CD8 subpopulations exist. Because of the different effector functions that they exert, it will be important to establish their relative contribution to anti-HIV immunity. Similarly, when studying immunity induced by HIV vaccines, it should be critical to account for the Granzyme B producing CD8 cell type as well, in addition to the conventionally studied IFN-g secreting CD8 cells.

V

Vaccines Against Allergy and Asthma

E1 **The Role of Mcl-1 in the Differential Regulation of Neutrophil and Eosinophil Apoptosis by Glucocorticoids**

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E1

The Role of Mcl-1 in the Differential Regulation of Neutrophil and Eosinophil Apoptosis by Glucocorticoids

Kelly L. Sivertson[1], Kristina K. Peachman[2], and David A. Bass[3]

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Circulating neutrophils and eosinophils undergo spontaneous apoptosis in the absence of activation/survival signals. Delayed apoptosis of these granulocytes is thought to be responsible for inflammation and eosinophilia in a number of inflammatory diseases, such as asthma. Addition of the glucocorticoid dexamethasone (dex), a common drug used to treat a broad range of inflammatory and autoimmune diseases, induces eosinophil apoptosis and delays neutrophil apoptosis. Previous studies show that the divergence in the steroid effects on these cell types occurs upstream of cytochrome c release from the mitochondria. Since the Bcl-2 protein family is vital to regulating the release of cytochrome c and other apoptosis mediators from the mitochondria, these proteins were likely candidates for the alternate effects in the neutrophil and eosinophil. Microarray studies were performed to identify apoptosis-related genes up- or down-regulated by dex treatment. Microarray results identified Mcl-1, an anti-apoptotic Bcl-2 family member, as a gene whose expression was altered by dex treatment. Mcl-1 contains PEST sequences typically found in proteins targeted for rapid degradation and has been shown to have a half-life of only 2 to 3 hours. The short half-life of Mcl-1 supports the theory that targeting of this gene for up- or down-regulation by dex would have rapid, but transient effects on the rate of apoptosis. Upon treatment with dex, Mcl-1 was shown by real-time PCR to be up-regulated in mRNA from neutrophils and down-regulated in mRNA from eosinophils. Western blots show that differences in levels of Mcl-1 protein in both cell types correlate with the changes in mRNA levels. These results suggest that the regulation of Mcl-1 plays an important role in the opposite effects of dex on neutrophil and eosinophil apoptosis.

9th National Symposium:
Basic Aspects of Vaccines

Sponsored by the
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30 April - 2 May 2003

NOTES

9th NATIONAL SYMPOSIUM

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